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ABSTRACT

This instructor's manual presents lesson plans for a unit of science within the Biomedical Interdisciplinary Curriculum Project (BICP), a two-year interdisciplinary precollege curriculum aimed at preparing high school students for entry into college and vocational programs leading to a career in the health field. Lessons concentrate on the circulatory system, with emphasis on the heart and blood vessels, blood and its properties, and the urinary tract. Designed to accompany the student text, lesson plans include objectives, recommended teaching time, and remarks. Keys to problem sets are also included. (CS)

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BIOMEDICAL SCIENCE

UNIT III

THE CIRCULATORY SYSTEM IN HEALTH AND SCIENCE

THE HEART AND BLOOD VESSELS;
BLOOD AND ITS PROPERTIES;
THE URINARY TRACT

INSTRUCTOR'S MANUAL
REVISED VERSION, 1976

THE BIOMEDICAL INTERDISCIPLINARY CURRICULUM PROJECT

SUPPORTED BY THE NATIONAL SCIENCE FOUNDATION

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INTRODUCTION

Unit III deals with the circulatory system and related health problems. The kidneys are included in this section because of their role in removing waste products from the blood and in regulating the concentrations of water and other substances in the blood. Respiration (Unit I), nutrition (Unit II) and circulation (this unit) are all interrelated. This unit emphasizes these relationships. For example, respiration brings oxygen to the lungs, where it is picked up by circulating blood. The blood transports oxygen to the cells of the body, where it is utilized in cellular metabolism.

Much of medicine is concerned with disorders of the heart, blood vessels and kidneys. In terms of human suffering and causes of death, the heart and blood vessels rank number one in the United States and in many other countries. Among the important medical problems we will consider in this unit are coronary heart disease, atherosclerosis, hypertension, anemia, edema, immunological disease and a variety of diseases of the urinary tract. Though some of these conditions may seem remote to students in their teens, the patterns of living that lead to these conditions are already being established in adolescence. In fact, it has been stated that coronary heart disease starts in puberty!

A. CONTENT OF THE UNIT:

This unit considers the heart and blood vessels, blood and its properties, and the urinary tract. A brief description of the three major segments of the unit follows.

PART I: THE HEART AND BLOOD VESSELS (Lessons 1 to 9)

The first two lessons introduce the unit and describe the kinds of blood vessels in the circulatory system. Students view circulation in a live goldfish in the laboratory. The third lesson reviews diffusion and osmosis. Lesson 4 deals with the anatomy of the heart and includes dissection of a heart. In lessons 5 and 6, the students learn how to measure blood pressure and heart rate. This is followed by lessons on cardiac output, electrocardiography and coronary heart disease risk factors.

PART II: BLOOD AND ITS PROPERTIES (Lessons 10 to 18)

The sequence of lessons on blood is introduced with a case study of iron-deficiency anemia, complemented in the laboratory by hemoglobin analysis of blood. This is followed by an introduction to the kinds of blood cells and a discussion of red blood cell functions. In the laboratory, students examine blood smears. Lesson 12 considers edema and its causes and the technique of electrophoresis is introduced in the laboratory. Electrophoresis of blood proteins is then presented in Lesson 13. Lessons 14 and 15 consider white blood cells and immunity. The process of blood clotting is treated in Lesson 16. The final lessons in this sequence review pH and discuss the buffers of the blood.

PART III: THE URINARY TRACT (Lessons 19 to 23)

Lesson 19 introduces the role of the kidneys and liver in purification and modification of the blood. In the laboratory, the students begin a series of activities on the effects of diet on the composition of urine. The text continues with a more detailed discussion of kidney function. Lesson 21 describes the importance of urinalysis and related techniques for studying the urinary tract. The last two lessons in this sequence deal with the artificial kidney, kidney transplants and diseases of the urinary tract. The corresponding activities involve the design and testing of an artificial kidney model.

FLOATING LESSONS: (Lessons X and Y)

Lessons X and Y are designed to be taught immediately after Math Lessons 15 and 17, respectively. These lessons are discussed under "Interdisciplinary Ties."

B. UNIT OBJECTIVES:

The student will

- list the five kinds of blood vessels in the circulatory system and the sequence in which blood flows through these vessels.
- trace the path of blood through the heart and lungs from the venae cavae to the aorta.
- state the function of the coronary arteries.
- explain how the following are used in medicine: electrocardiograph, electrophoresis, sphygmomanometer, stethoscope.
- state what happens to blood pressure and the rate of blood flow as blood moves from the heart through a complete cycle of the circulatory system.
- state what the following waves in the electrocardiogram represent: P,QRS complex, T.
- state the functions of red blood cells, white blood cells and platelets.
- state how the red blood cell is adapted to its functions.
- define edema and list at least three causes of this condition.
- list at least one disease detectable by blood smears, and one detectable by clotting time.
- explain how the following are interrelated: immunity, antigen, antibody, lymphocyte.
- compare and contrast passive and active immunity.
- state the relationship of platelets and fibrin to blood clotting.
- list at least three important blood buffers.
- explain how a CO_2-bicarbonate buffer resists a pH change when an acid or base is added.
- state at least two different ways in which the kidneys contribute to homeostasis.
- list the parts of the urinary tract.
- describe at least two disorders of the urinary tract.
- list at least two substances commonly determined in urinalysis and the use of such analyses.
- outline how an artificial kidney operates.

This volume contains teacher materials for a total of 25 lessons. A Student Text and Laboratory Manual are available in separate volumes.

C. INTERDISCIPLINARY TIES:

1. Biomedical Mathematics: The following ties between Science and Mathematics deserve special consideration. Additional relationships are suggested in individual lessons, and you and your colleague teaching Biomedical Mathematics may discover still other relationships.

a. Chemical equilibria and solubility products are discussed in Mathematics Lesson 12. Related topics were discussed in Science Unit II, Lessons 27 and 28. A discussion between teachers in the two disciplines would be fruitful. You may wish to review related Science concepts when Mathematics Lesson 12 is being taught.

b. Science Lessons 17 and 18 consider pH and buffers, while Math Lessons 14 through 19 review pH and dissociation in a more quantitative way. It would be extremely helpful for you and the Biomedical Mathematics instructor to coordinate

your plans for these lessons. It would be ideal to have Science Lessons 17 and 18 follow very shortly after Mathematics Lesson 14, since the Mathematics lesson reviews pH. Equilibrium concepts are tested in the laboratory in Science Lessons X and Y. These are floating lessons intended to be used shortly after Math Lessons 15 and 17, respectively. Note that the Mathematics sequence is treated as optional. Consequently, Science Lessons X and Y should not be taught unless the related Mathematics lessons are taught. It is essential for you and the Biomedical Mathematics instructor to coordinate your plans with regard to these lessons.

2. Biomedical Social Science: Two obvious ties between Science and Social Science are discussed below. Additional relationships are discussed periodically in the Instructor's Manual.

a. The subject of artificial heart implants (from the standpoint of decision-making about allocation of a scarce resource) is considered in Social Science Lessons 1 through 7. Though the material is not treated from the technical standpoint, it would be desirable for the students to be studying the heart and the circulatory system in Science at about the same time that the artificial heart allocation problem is being considered in Social Science. Lessons 4 through 9 in Science are the most relevant (in that they deal with the heart) so if the students begin Science Unit III at the same time or a little before they begin Social Science Unit III there should be a good fit.

b. Science Lesson 9 considers the factors that are associated with coronary heart disease (CHD). In Social Science Lessons 18 through 21 students will be designing posters to encourage young people to avoid the risk factors for CHD. Although the Social Science lessons consider CHD in a very different context than the Science lesson, it is important for Science Lesson 9 to precede the Social Science sequence. The gap between the Science and Social Science should not be more than two weeks.

c. In Social Science Lessons 12 through 17 students will select public issues, gather information on them and analyze them in terms of social-science concepts related to government. Many government-related issues are also related to health, and some are directly related to diseases of the circulatory system and kidneys. During the first weeks of Unit III you and/or the students should keep an eye out for local, state or national issues that involve both (1) government on any level and (2) health or health care, particularly the prevention or treatment of heart, blood and kidney diseases. Pass your suggestions to the students or to the Social Science instructor before Social Science Lesson 12 is taught.

D. CAREER INFORMATION:

Biomedical Science Unit III has many career implications, and field trips to visit specialists or visits by health professionals can be a worthwhile supplement. To make the best use of outside speakers, the following table may be useful.

Specialist	Relevant Lesson(s)
Anatomist	1, 2, 4, 20, 23
Physiologist	2, 3, 6, 7, 8, 12, 15, 19
Cardiologist	4, 5, 6, 7, 8, 9
ECG technician	8, 9
Epidemiologist	9
Biostatistician	9
Hematologist	10, 11, 14, 16
Medical technologist (or Laboratory technician)	10, 11, 13, 21
Biophysicist	2
Cell biologist	3, 10, 11, 14, 16
Nurse practicioner	10
Dietician	9, 10, 12
Developmental biologist	11

Biochemist	13, 16, 20
Immunologist	14, 15, 22
Microbiologist	15, 23
X-ray technologist	9
Chemist	17, 18, X, Y
Urologist	19-23
Endocrinologist	19, 20
Biomedical engineer (or Biomedical electronics technician)	8, 22
Blood bank technologist	10, 11
Renal dialysis technician	22
Urology technician	22, 23
Cytotechnologist	10, 11

E. LABORATORY ACTIVITIES REQUIRING ADVANCE PLANNING:

1. Live goldfish are needed for LA-2. They should be obtained a few days in advance of the activity.

2. For the dissection activity in LA-4, fresh rather than preserved animal hearts (calf, pig, beef or sheep) are the best choice. It is a good idea to contact a butcher or slaughterhouse in advance of the lab.

3. Stethoscopes and sphygmomanometers are needed for LA-5. It may be possible to borrow a few instruments by contacting the school nurse or your community hospital in advance of this activity.

4. Live daphnia are required for LA-7. They should be ordered so as to arrive a few days in advance of the activity. They may be kept at room temperature without an aerator, for about a day. For longer storage, an aerator would be helpful.

5. An oscilloscope is optional but very useful in LA-8. You may be able to borrow one or more from your colleagues in physics or electronics. If you are unfamiliar with the operation of the available oscilloscope(s), it would be worthwhile to practice the technique before the activity.

6. LA-10 and several other activities that follow call for finger punctures. Check in advance on any local or state regulations regarding taking blood via finger punctures from students. For example, parental permission may be required in some communities.

7. In LA-11, there is an option requiring slides of abnormal blood cells. You may be able to obtain some sample slides by contacting a nearby hospital laboratory.

8. Activity 14 calls for duplication of materials provided in the Instructor's Manual. You may wish to assign this to a student assistant.

F. BIBLIOGRAPHY:

Almost any reference book on the circulatory system and kidneys will be useful for review purposes. Several sources were cited in the Unit II Instructor's Manual, p. viii. Of these, the textbooks by the following authors would also be useful in this unit:

Keeton; Langley; Pansky; De Coursey; Landau.

In addition, the following were among the references used in writing the unit.

W.V. Brown and E.M. Bertke, Textbook of Cytology, C.V. Mosby Co., St. Louis, 1974.

L.M. Elson, It's Your Body, McGraw-Hill Book Co., N.Y., 1975.

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L.W. Diggs, D. Sturm and A. Bell, The Morphology of Human Blood Cells, Abbott Lab, Chicago, 1970.

A.J. Vander, J.H. Sherman and D.S. Luciano, Human Physiology, McGraw-Hill Book Co., N.Y., 1970.

I. Davidsohn and J.B. Henry, Todd-Sanford Clinical Diagnosis, W.B. Saunders Co., Philadelphia, 1969.

N.W. Tietz, Ed., Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia, 1970.

G. GENERAL SUGGESTIONS:

1. You may wish to provide bulletin board space for newspaper clippings related to the circulatory system and the kidneys. Materials collected may be of general interest and can be used in appropriate lessons.

2. It should be possible to obtain brochures and films from your local Heart Association.

3. Unit III is the last unit of the first year's curriculum. Consider where you are with regard to the school calendar so that you can pace the teaching of the unit. If you are pressed for time, some of the activities could be shortened and some text could be eliminated at your discretion. If necessary, the kidney sequence could be taught in the following year, but the beginning of the second year requires an early start on Unit IV materials because of Mathematics-Science ties.

4. Your team should consider year-end activities that will make the curriculum more meaningful to the students. It might be possible for you and your students to plan some kind of community or school health project such as an exhibit or blood drive or health survey.

LESSON 1: (A) INTRODUCTION TO UNIT

(B) PATHWAY OF BLOOD FLOW

RATIONALE:

This lesson introduces the entire unit on the circulatory system. In addition, it is the first of nine lessons on the heart and blood vessels. This lesson presents the anatomical and physiological concepts basic to an understanding of the heart, blood vessels, and blood. In the succeeding lessons these areas are explored in more detail. A knowledge of the basic anatomy of the circulatory system is necessary to consider atherosclerosis and coronary heart disease which will be treated in later lessons as well as the normal functioning of the circulatory system under different conditions.

OBJECTIVES:

The student will:

- identify three functions of the circulatory system.
- list and distinguish between the five kinds of blood vessels.
- identify on a diagram and trace in proper sequence the names of the following structures in the pathway of blood flow in humans: heart, lungs, artery, arteriole, capillary, venule, vein.

SEQUENCE: ST-1; Activity 1

SUGGESTIONS:

1. You may wish to begin the first lesson of Unit III by discussing the contents of the unit, some of the health problems to be considered, related health careers, etc. as discussed in the introduction to the unit.

2. The following question may be helpful to start a discussion. "What happens to the cells in a section of the body if blood flow to that section is partially blocked?" (Answer: The cells no longer get enough of the materials they need [i.e., oxygen, nutrients] and no longer get rid of sufficient wastes, resulting in either impairment of function or death of the cells.)

3. You may wish to have students calculate how many liters of blood the heart pumps in 10 years, providing the information that the heart pumps about 70 ml of blood with each beat, and beats an average of 70 times per minute, at rest. Supply the conversion factor .264 liter = 1 gallon, if they wish to convert the result to gallons. Similar calculations were performed in Math Unit I, Problem Set 36.

$$70\ \frac{\text{ml}}{\text{beat}} \cdot 70\ \frac{\text{beats}}{\text{minute}} \cdot \frac{\text{liter}}{1000\ \text{ml}} \cdot 60\ \frac{\text{minutes}}{\text{hour}} \cdot 24\ \frac{\text{hours}}{\text{day}} \cdot 365.25\ \frac{\text{days}}{\text{year}} \cdot 10\ \text{years} \approx$$

$$\approx 2.6 \times 10^7 \text{ liters } (\approx 6{,}800{,}000 \text{ gallons})$$

4. You may wish to use the data in the Information on Circulation for discussion. The following points may be brought out:

a. Students could compare the percentage of blood flow per minute to each region with the mass of the region. It will be seen that the kidneys have the second largest flow, yet a very small weight. This is consistent with the fact that the kidneys have a vital role in continuously regulating the blood: its salt concentration, volume, and other characteristics.

b. Students could compare the percentage of total blood flow to each region. Whenever the blood flow is high compared to the oxygen consumption, it may be suspected that the blood is there for reasons in addition to supplying oxygen and nutrients and carrying away waste products. This is seen in the cases of the liver, kidneys, and skin. In the skin blood is cooled. The liver processes nutrients from digestion and the products of cell metabolism. Both the nutrients and the

products of cell metabolism are carried to the liver by the blood. The role of the kidneys was described above.

c. Students could compare the relative rates of oxygen consumption per mass of each region. Point out that the heart has the highest oxygen consumption per mass. This underscores the critical nature of a good coronary circulation and correlates with the fact that the heart muscle constantly contracts. Such activity requires energy. Oxygen is necessary to meet long-term energy requirements. The kidneys too have a high oxygen consumption. This correlates with the fact that they require much energy to process the Na^+ and other ions in the process of making urine (active transport).

INFORMATION ON CIRCULATION:

Blood flow to and oxygen consumption by the various organs at rest is given in the table below. It may be used to supplement Table 2 of Activity 1.

TABLE: BLOOD FLOW AND OXYGEN CONSUMPTION AT REST

REGION	PERCENTAGE OF TOTAL BODY MASS	PERCENTAGE OF TOTAL BLOOD FLOW PER MINUTE	PERCENTAGE OF TOTAL OXYGEN CONSUMPTION	RELATIVE RATE OF OXYGEN CONSUMPTION PER MASS OF REGION (COMPARED TO THE WHOLE BODY)
Liver	4.1	27.8	20.4	5.0
Kidneys	0.5	23.3	7.2	15.0
Brain	2.2	13.9	18.4	8.3
Skin	5.7	8.5	4.8	0.8
Heart muscle	0.5	4.6	11.6	24.3
Other muscle	49.2	15.6	20.0	0.5
Rest of body	37.8	6.3	17.6	0.5
Whole body	100.0	100.0	100.0	1.0

INFORMATION ON ACTIVITY 1:

TEACHING NOTES:

1. The purpose of this activity is to acquaint students with the basic anatomy and physiology of the circulatory system, including the role of the lungs, heart, and the different kinds of blood vessels.

2. Anticipated time: one period.

3. A preserved fetal pig or cat may be useful. The organs mentioned in the activity can be exposed to view, or students may dissect and look for the organs and blood vessels. Models and/or diagrams of the circulatory system may be useful in the same way.

4. This activity does not consider why the pulmonary artery is classed as an artery even though it carries blood low in oxygen and high in carbon dioxide. You may wish to discuss this point with the class--this vessel is an artery because it carries blood from the heart. The amount of oxygen in the blood does not determine whether a vessel is an artery or a vein.

LESSON 2: ARTERIES, VEINS AND CAPILLARIES

RATIONALE:

The structures of arteries, veins and capillaries correlate with the different roles they play in the transport of blood. Abnormal alteration in their structures may result in a variety of circulatory problems. ST-2 examines and compares the blood vessels and how they change in response to stresses to the body. In LA-2, blood circulating through vessels is observed directly in a goldfish.

OBJECTIVES:

The student will:

- compare the diameters of arteries, capillaries and veins.
- state the function of the thin walls of capillaries.
- state what happens to blood pressure and the flow rate of blood as blood moves through the circulatory system.
- state the function of valves in veins and how weak valves result in varicose veins.
- give two examples of phenomena during which blood vessel diameter changes.
- distinguish between arterioles, venules, and capillaries in the tail of a live goldfish.

SEQUENCE: ST-2; LA-2

SUGGESTIONS:

1. These discussion questions may be useful.

a. When a vein or an artery is cut, the loss of blood can be reduced by applying pressure at an appropriate point. Where should it be applied for a cut vein in the upper arm? Where should the pressure be applied for a cut artery in the wrist? Explain your answers. (In both cases the pressure must be applied "upstream" between the cut and the elbow.)

b. What happens to an organ if an artery to the organ is blocked? (Answer: Blood supply to the organ through that artery stops and the part of the organ supplied by the artery dies, unless it is also supplied by a second artery.)

c. What would happen if a vein from an organ were blocked? (Answer: Blood would back up into the organ and the organ would swell up. This is far less common than a blocked artery. Atherosclerosis commonly blocks arteries but rarely occurs in veins.)

2. You may wish to present the graph in the figure given in the Information on Blood Vessels and relate it to the fact that blood spurts out from cut arteries and only dribbles out in veins.

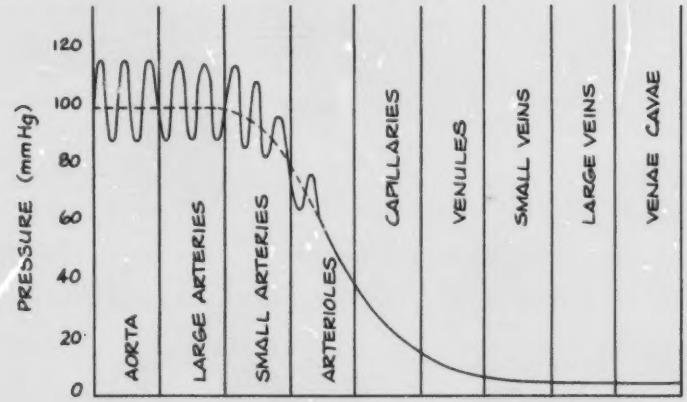
3. The table in the Information may be used to present the fact that capillaries have the largest total cross-sectional and surface area in the body even though they are the smallest vessels. A pattern exists in which, in general, the smaller the vessel, the more there are, much like the branches of a tree.

4. It may be a good idea to remind students that they have heard about valves before--in regard to the digestive tract. In both systems (digestive and circulatory) valves perform the same function. They permit materials to move in only one direction.

5. You may wish to discuss how hyperventilation produces dizziness. See Information on the following page.

INFORMATION ON THE BLOOD VESSELS:

It is perhaps startling to learn that at any moment during rest over 50 per cent of the blood is in the veins and only about 15 per cent in the arteries. The various vessels are listed in the figure; blood passes through each kind in turn as it circulates. Notice that blood pressure drops in each successive kind of vessel, the greatest drop occurring in the arterioles.



Blood pressures in the different portions of the circulatory system, excluding the lungs.

The table below shows data on the different blood vessels. (Although the aorta is an artery and the vena cava is a vein, they are listed separately.) The table lists the vessels, their total cross-sectional area and other information. The total cross-sectional area is obtained by multiplying the total number of each type of vessel by its cross-sectional area. Notice that although capillaries are the smallest in diameter, they have the largest total cross-sectional area. This is because there are so many of them (about 16 billion in this table).

	Internal diameter	Approximate Total Cross-sectional area, sq cm	Percentage of Blood Volume Contained*	Total Number
Aorta	2.5 cm	4.9	2	1
Artery	0.5 cm	20	8	~100
Arteriole	30 μm	400	1	57 million
Capillary	6 μm	4500	5	16 billion
Venule	20 μm	4000	54	1.3 billion
Vein	0.5 cm	40		~200
Vena cava	3 cm	14		2

*In blood vessels not including the heart and lungs.

Arterioles: The Major Blood Flow Regulators

Arterioles are the most important regulators of blood flow. They are much smaller in diameter than arteries, with 1 to 5 layers of muscle in their walls.

It is an interesting physical property that the rate of flow through narrow tubes varies directly with the fourth power of the radius. Arteries enter an organ and then branch into arterioles. Thus, if the arterioles in an organ constrict to half their former radius, flow in the organ becomes $(\frac{1}{2})^4$ or $\frac{1}{16}$ as fast. Similarly, if the arterioles dilate to twice their former radius, blood flow increases by 2^4 or 16-fold. Since each arteriole supplies 10 to 100 capillaries, this means that a

small change in an arteriole's radius greatly alters capillary blood flow. Such vessels change their radius when the muscle cells in their walls contract or relax.

How Vessel Diameter Changes

During exercise, the brain sends impulses down particular nerves which connect to muscle cells in all vessel walls except capillaries (capillary walls lack muscle cells). These impulses cause the arterioles and small arteries in the kidneys and digestive tract to constrict, while the arterioles and small arteries in the muscles used in exercise dilate. This causes more blood flow through exercising muscles.

Even more important than nerve effects on flow rate is local regulation: the lack of oxygen, increase in temperature, and possibly the extra CO_2 and lactic acid produced during exercise, directly cause arterioles in working muscles to dilate. In addition, epinephrin (adrenalin) secreted by the adrenals into the blood during exercise helps to regulate the rate of blood flow. Blood vessels in muscles respond to epinephrin by dilating and those in the kidneys respond by constricting.

Acting in harmony, the heart rate and the force of the heart's contraction increase, forcing more blood into the muscles. All these effects together can cause up to a 20-fold increase over the resting state in blood flow through skeletal muscles.

Hyperventilation

Hyperventilation was discussed in Unit I. It causes dizziness because it makes more CO_2 leave the blood in the lungs, which lowers the CO_2 content of blood. When this blood reaches the brain, the vessels there respond by constricting. This reduces blood flow to the brain, causing dizziness.

This homeostatic system works to keep the CO_2 level of blood in the brain at a constant level. If the CO_2level gets too high, as after a heart attack or severe respiratory illness, the vessels in the brain dilate, effecting an increase in blood flow in the brain, which lowers the CO_2 level of the brain's blood. On the other hand, if the CO_2 level of the blood gets too low, as during hyperventilation, the vessels constrict, which acts to raise the level back to normal.

By holding the CO_2 level constant, this system also acts to maintain the right level of blood flow. This insures a proper supply of oxygen and proper removal of other wastes.

Blood Pressure:

The blood pressure in the aorta is at its maximum when it is most full of blood. This occurs while the heart is pumping blood into it. At this point, the blood pressure is typically between 100 and 140 mm Hg. As the aorta recoils to squeeze blood out, the pressure drops, but not to zero, for before the aorta can finish squeezing, the heart pumps more blood into it. The lowest pressure reached in the aorta is between 60 and 90 mm Hg in most people.

These pressures are the common clinical measures of the blood pressure in the aorta. They are virtually the same in arteries in the arm, where blood pressure is actually measured. Blood pressure will be presented in detail in later lessons.

INFORMATION ON LABORATORY ACTIVITY 2:

TEACHING NOTES:

1. The purpose of this activity is to provide the student with an opportunity to observe blood flow in a living animal.

2. Anticipated time: one period (up to three periods if all options are elected).

3. Fish may be kept in large jars instead of aquaria. You may wish to arrange for students to take the fish home after the activity.

4. The procedure calls for the 10X objective only. It may be best to use only

the 10X (and, if available, a lower power objective,) to avoid getting the 40X objective wet. Aquarium water is dirty, and if it dries on an objective, a layer of dirt may remain which is difficult to remove. You may wish to remind students how to clean an objective.

5. Two optional activities are given below. They are written in the form of instructions to students and may be reproduced for classroom use. In option B, a tourniquet may be used if there aren't enough students with protruding arm veins. In this case, use a gauze or cloth strip and tie it comfortably around the arm. Untie every 5 to 10 minutes to give the arm a rest.

MATERIALS: (for 15 set-ups)

- fish net
- 2 aquaria--one labeled "fresh fish" and one labeled "recovery room"
- 20 to 25 live goldfish
- 15 Petri dish covers or bottoms or combination (plastic or glass)
- 15 pieces of cotton fabric, about 8 cm by 2 cm
- 15 microscope slides
- 15 strips of gauze (12 to 20 cm long, 3 to 5 cm wide)
- 15 small jars or beakers of aquarium water
- 15 medicine droppers
- 15 microscopes
- tape
- lens paper

ANSWERS TO PROCEDURE QUESTIONS:

1. Blood flows toward the heart in a venule and the opposite way in an arteriole.

2. Blood flow in arterioles is probably pulsating.

3. The heart pumps blood in spurts.

4. Smooth. By the time blood arrives in veins, the pulsing has been dampened by the capillaries.

5. Flow is irregular, as blood cells become stuck in the constriction between arteriole and capillary and then freed at irregular intervals.

6. a. Direction of flow.

   b. Flow in spurts in arterioles.

   c. Arterioles slightly thinner than venules.

ANSWERS TO DISCUSSION QUESTIONS:

1. Oxygen in the water enters its blood through capillaries in its gills. The blood circulates to its tail in blood vessels.

2. Narrower.

3. It enables more blood to flow through the muscles to meet their needs for oxygen and nutrients.

When skin arterioles narrow, less blood flows in the skin, so less heat is lost to the air.

ANSWERS TO QUESTIONS IN OPTIONAL PROCEDURE A:

1. Muscle cells in arteriole walls enable arterioles to constrict or dilate.

2. and 3. Dilate. This permits more blood to flow through the muscles, to supply them with the additional oxygen and nutrients they require to work.

4. a. Veins constrict, due to the muscle cells in their walls. This squeezes the extra blood that is inside them so that it flows back to the heart.

b. Valves permit movement in only one direction.

5. Heart, arteries, arterioles, capillaries, venules, veins, heart.

A. COMPARISON OF BLOOD VESSEL CROSS SECTIONS

INTRODUCTION:

The arteries, veins and capillaries have different functions and different pressures within them. They also have different structures, which are related to their different functions. This activity focuses on the structural differences.

MATERIALS:

prepared slide of cross section of artery and vein

microscope

PROCEDURE:

1. Look at the slide preparations and answer the following questions. The slide contains cross sections (abbreviated "cs" or "xs") of an artery and a vein. The cross sections are thin slices of vessels, made by cutting them at right angles to their length, just as you would slice a salami or loaf of bread (Figure 1).

FIGURE 1: Appearance of cross sections.

2. Place the slide on the stage of the microscope and focus with the low power (10X) objective on the walls of the artery and then the vein. The artery should appear round in comparison to the vein. The vein may appear somewhat flat. You may be able to see blood cells in the central region.

You should see three layers in the wall of each vessel. The inner layer is a thin layer of cells called endothelium. Endothelium is a special tissue which lines many organs of the body. The middle layer is composed of smooth muscle cells with elastic fibers mixed in. The outside layer is composed of connective tissue. Connective tissue is found throughout the body. The various tissues that support, bind or protect are all connective tissues.

3. Compare the wall of the artery with the wall of the vein. Note the thickness of each layer in the wall. Record your observations.

4. The smallest arteries are called arterioles. As with other vessels, the muscle cells in the wall make the arterioles capable of being "constricted" or "dilated." Constriction is the narrowing of the diameter of the vessel, and dilation is the enlargement of the diameter of the vessel.

What function does the muscle action enable the arteriole to perform? (1) When you run hard, would it be better for the arterioles in your legs to constrict or dilate? (2) Explain your answer. (3)

5. Like small arteries, veins can constrict. When veins constrict, the blood inside them is squeezed. This causes more blood in the veins to flow back to the heart.

During exercise, the amount of blood entering the veins from capillaries increases because the heart is pumping more blood into vessels. State one way in which the veins help keep the additional blood moving back to the heart. (4)

6. Arteries branch into arterioles. The arterioles branch into capillaries; the capillaries rejoin into venules. Venules are the smallest veins in the body. They join into veins, which return to the heart.

List in proper sequence the flow of blood from the heart through the different kinds of blood vessels and back to the heart. Start and end with the heart. (5)

B. THE STRUCTURE OF VEINS

INTRODUCTION:

The structure of veins helps them to return blood to the heart. For example, the veins in your arms and legs are surrounded by skeletal muscles. When you move, you use these muscles, and the muscles exert pressure on the veins. This causes blood to flow through the veins. In addition, the walls themselves contain muscle cells--these muscle cells can contract in a way that causes the veins to become narrow. This constriction squeezes the blood toward the heart. The blood must move in this direction because the veins have valves which permit movement in one direction--toward the heart. One other factor which is not related to the structure of veins helps the blood to return to the heart. It is gravity. When you are sitting or standing up, gravity helps blood to flow from the brain downward through veins to the heart.

PROCEDURE:

To see valves, find a person with protruding arm veins. Perform the demonstration that William Harvey did back in the 1620's.

a. The position of each valve is indicated by a swelling or knot, B,C,D,E. (See Figure 2.)

b. The blood is pressed out of a vein from H past O with one finger, while another keeps the vein closed at H. No blood runs back past the valve at O.

c. If the vein is pressed by another finger at K, no blood can be forced backwards past the valve at O.

d. The vein is closed with one finger at L, as before, and emptied by stroking with another finger, M, towards the valve at N; the vein remains empty until the finger at L is removed, when it rapidly fills up from the hand.

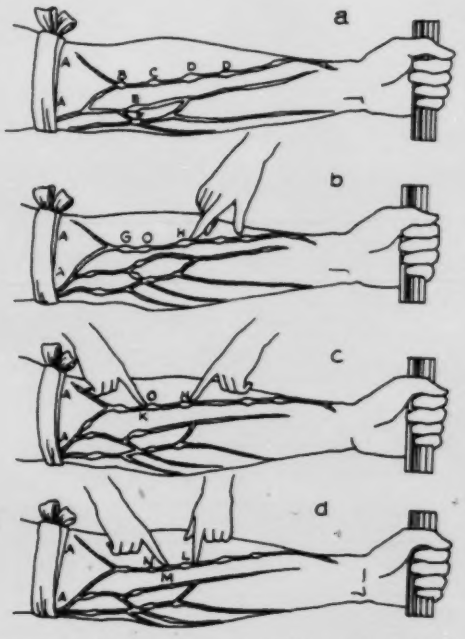


FIGURE 2: Demonstration of valves in arm veins.

LESSON 3: REVIEW OF DIFFUSION AND OSMOSIS

RATIONALE:

The preceding lessons presented the pathway of blood flow and viewed the importance of capillaries. This lesson focuses on how the water and dissolved substances move from the capillaries into the interstitial space. The processes of diffusion and osmosis explain this movement, and they also explain how liquid returns to the bloodstream from the intracellular fluids. The concepts of diffusion and osmosis were developed in Unit I in the treatment of gas exchange in the alveoli and pulmonary capillaries. However, it is likely that this review will be necessary. Furthermore, diffusion and osmosis are basic to the treatment of edema and the kidneys later in this unit.

OBJECTIVES:

The student will:

- define the terms cell membrane, permeable and semipermeable.
- explain, in terms of the concentration of water molecules, why water moves across a membrane from a region low in protein to a region high in protein.
- list the two chemical components of cell membranes.
- explain how water outside a capillary re-enters the capillaries.
- explain what would happen if a cell were placed in distilled water.

SEQUENCE: LA-3; ST-3

SUGGESTIONS:

1. You may wish to set up a demonstration of osmosis.

2. The cell membrane is discussed in Section 3-2. You may wish to elaborate on some of the newer theories of the structural nature of the cell membrane. Most recent cell biology texts treat this subject in detail. For review, you could refer to Brown and Bertke, Textbook of Cytology, 2nd Ed., C. V. Mosby, St. Louis, 1974 (Chapter 6) or De Robertis, et al, Cell Biology, 5th Ed. W. B. Saunders Co., Philadelphia, 1970 (Chapter 11). You may wish to review the chemical nature of fats and proteins.

3. It might be interesting to project a few slides or overhead transparencies of electron micrographs of cells. Have the students point out the cell membrane and the nucleus.

4. You may wish to extend the treatment of diffusion to include the fact that there are two ways in which substances diffuse through capillary walls: (1) fat-soluble substances (such as lipids), O_2, CO_2, and alcohols, pass right through the capillary walls, (2) water-soluble substances, including water, salts and sugars only pass through thin spaces in the walls between adjacent endothelial cells. This is a slower process than the first.

KEY REVIEW SET 3:

1. arteries
2. true
3. veins
4. true
5. true
6. capillaries
7. true
8. arterial, venous
9. true
10. arterioles
11. true

INFORMATION ON LABORATORY ACTIVITY 3:

TEACHING NOTES:

1. The purpose of this activity is to reinforce the concepts of diffusion and osmosis.

2. Anticipated time: one period (not including options that follow).

3. The Respiration Unit had two activities (Laboratory Activities 33 and 34) on diffusion and osmosis. If these activities were not done, they may be used as possible optional activities or demonstrations. There is an additional option in the teaching notes of LA-34, Unit I.

4. Do not leave egg set-ups overnight for use the next day. The egg white will dry in the pipet and clog it.

5. A 1-ml pipet may be used in place of a 10-ml one. This will cause the fluid level to rise rapidly, so fast it may flow out the top in less than one period.

MATERIALS: (for 15 set-ups)

PART I:

20-24 chicken eggs

15 10-ml pipets
(or 50-cm glass tubes)

15 beakers, 250-ml (or larger)

15 ring stands and clamps

15 clamps

silicone sealant, or candle wax and matches

5 to 10 pointed instruments (scissors or knives with sharp points

5 to 10 mm rulers

PART II:

15 slides	15 dropper bottles (for tap water)
15 coverslips	15 dropper bottles of .85 M NaCl
15 microscopes	lens paper
1 to 2 tomatoes	paper towels or other tissue

PREPARATION OF REAGENTS:

0.85 M sodium chloride solution, 100 ml: 5.0 ± .2 g NaCl plus sufficient water to make 100 ± 2 ml.

ANTICIPATED RESULTS:

PART I:

At first, no water is seen in the tube; later water is seen rising in the tube very slowly.

PART II:

The tomato cells swell up (as water enters the cells). The cells do not burst. (This is because of rigid cell walls in plants.)

ANSWERS TO DISCUSSION QUESTIONS:

1. The fluid level rose inside the pipet.

2. It swells up.

3. Tap water has a higher concentration of water than the solution inside the egg. By osmosis, water moved through the eggshell and membrane underlying the shell. When the space inside the egg was filled with water, further osmotic pressure forced water to move up the tube.

4. No. The fluid outside the cell has a higher concentration of water than the fluid inside, so water enters the cell.

5. Diffusion is the movement of any substance from a region of high concentration to a region of low concentration. Osmosis is the movement of water (or other solvent) through a semipermeable membrane, from high to low concentration.

LESSON 4: (A) THE HEART: STRUCTURE AND PATHWAY OF BLOOD THROUGH IT

(B) DISSECTION OF THE HEART

RATIONALE:

During upcoming activities on heart sounds and the electrocardiogram, the student will study the heart's action. The anatomy of the heart and the path of blood flowing through it are presented now in preparation for this. Knowledge of heart structure prepares the student for the study of how the heart functions during exercise and disease, presented in the next several lessons. An understanding of heart anatomy is necessary for an understanding of heart disease.

OBJECTIVES:

The student will:

- identify the following structures on a diagram or model of a four-chambered heart: atria, ventricles, pulmonary artery, pulmonary veins, aorta, venae cavae, and valves.

- state the functions of the structures listed above.
- name in correct sequence the chambers, arteries and veins as blood moves through the heart and lungs.
- dissect a four-chambered heart and identify the structures listed in the first objective above.

SEQUENCE: ST-4; LA-4

SUGGESTIONS:

1. Diagrams, charts and/or models of the heart would be helpful in the presentation of this lesson.

2. You may wish to have students draw in arrows on a heart diagram as a review of the path of blood flow as shown in Figure 1 of ST-4. Figure 2 of LA-4 might be copied and distributed for this purpose.

3. Different books may contain different names for the heart valves. Atrioventricular valves = A-V valves. There are two A-V valves: the left one is sometimes called the mitral valve or bicuspid valve; the right one is also known as the tricuspid valve. There are two semilunar valves. The right one is the pulmonary valve. The left one is the aortic valve.

4. It may be helpful to explain the orientation of ST-4, Figure 4. It is a view from above and behind the heart.

INFORMATION ON LABORATORY ACTIVITY 4:

TEACHING NOTES:

1. The purpose of this activity is to familiarize students with the anatomy of the heart and also to help students develop skills in dissection.

2. Anticipated time: one to two periods.

3. Calf, pig, beef or sheep hearts can all be used for this activity. If these are unavailable, a chicken or turkey heart (although smaller) can also be used.

4. If there aren't enough hearts for the entire class, some students may proceed to read ST-5 and LA-5 and perform dissection the second day on the used hearts. In this case, tell the students who dissect on the first day to preserve the tissues of the heart as much as possible--not to destroy structures.

5. If you purchase the hearts fresh, ask the butcher or slaughterhouse not to cut the chambers open. Fresh hearts can be frozen until needed.

6. If you purchase preserved hearts be sure to soak them in water at least 48 hours before use to remove most of the preservative.

7. Preserved hearts which may have been dissected in other classes (biology courses, etc.) at other times can be used in this activity. Modify the procedure to fit the condition of the hearts you plan to use.

8. Blunt probes may be glass rods or similar items.

MATERIALS: (for 10 set-ups)

- 10 four-chambered hearts
- 10 blunt probes
- 10 scissors, dissecting
- 10 razor blades or scalpels
- 10 dissecting pans
- model and/or diagrams of the heart (optional)

ANSWERS TO PROCEDURE QUESTIONS:

1. The sac protects the heart. The fluid keeps the heart tissues moist and acts as a lubricant thereby permitting the heart to slide with minimal friction when it beats.

2. The upper chambers are smaller and have thinner and more flexible walls.

3. The wall of the left ventricle is thicker.

4. Atrium to ventricle.

5. The cords keep the valve flaps from reversing their direction, which would let blood flow backward.

6. A-V valve.

7. To the lungs.

8. To all arteries except the ones that go to the lungs.

9. The aortic valve is thinner and smaller.

10. It prevents blood in the aorta from flowing back into the heart.

11. Away.

12. The heart.

13. Blockage would cut off blood supply.

14. The ventricle's walls are thicker.

15. Ventricles exert much more pressure on blood, to pump it into the arteries. Atria pump blood only into the ventricles, which requires less pressure.

16. The lungs.

17. Pulmonary veins.

18. Pulmonary arteries carry blood low in oxygen and high in CO_2. Pulmonary veins carry blood high in oxygen and low in CO_2.

19. Vessels entering atria are thin-walled and flabby compared to vessels leaving ventricles. The former are veins, the latter arteries.

20. venae cavae, right atrium, A-V valve (tricuspid), right ventricle, pulmonary valve (semilunar), pulmonary artery, lungs, pulmonary veins, left atrium, A-V valve (mitral), left ventricle, aortic valve (semilunar), aorta, body (arteries, arterioles, capillaries, venules, veins), venae cavae.

KEY TO PARTS OF HEART SHOWN IN FIGURE 2:

1. vena cava
2. right A-V valve
3. pulmonary artery
4. pulmonary veins
5. left A-V valve
6. aorta
7. septum
8. right atrium
9. left atrium
10. right ventricle
11. left ventricle
12. pulmonary valve
13. aortic valve
14. vena cava

LESSON 5: HEART SOUNDS, BLOOD PRESSURE AND PULSE BEAT

RATIONALE:

This sequence on the heart so far has covered the anatomy and path of blood flow through the heart and vessels. Now this information is used to understand some important clinical tests for circulatory function, including the arterial pulse, the arteriolar pulse, heart sounds, and blood pressure. The laboratory activity gives students the opportunity to perform these tests and observe changes that occur in the function of the heart and blood vessels during exercise.

OBJECTIVES:

The student will:

- state three conditions that increase the pulse rate.
- explain the advantage of an increase in pulse rate during exercise.
- explain, in terms of valve closings, how the two main heart sounds are made.
- explain how a valvular defect may cause a heart murmur.
- state what a health professional listens for through a stethoscope over the heart.
- take a pulse rate.
- use a stethoscope to listen to heart sounds.
- use a sphygmomanometer to measure blood pressure.
- demonstrate ballistocardiography.

SEQUENCE: ST-5; LA-5

SUGGESTIONS:

1. A guest speaker to talk about heart-monitoring instruments may be of interest. You might ask the school nurse to demonstrate how to use the stethoscope and sphygmomanometer.

2. An understanding of how each heart sound is made calls for a careful study of the sequence of valve openings and closings during the heartbeat. You may wish to refer to the Information on Heart Sounds and Pulses.

3. The answer to the problem posed in Section 5-3 on the direction in which the artificial caged ball valve would be inserted into the aorta is, "with the ring part toward the left ventricle." This way, blood squeezed from the ventricle pushes the ball away from the ring allowing blood to exit. Blood flowing back pushes the ball into the ring to make a seal which prevents backflow.

4. You may wish to discuss in detail what makes valves open and close. See Information below.

5. Students might find it interesting to listen to abnormal heart sounds. Kits that cost $2.30 each and include actual recordings of heart sounds and a booklet on their interpretation may be obtained by requesting an "Interpreting Heart Sounds" kit from Interpreting Heart Sounds, c/o Hoffmann-La Roche Inc., Box 1577, Newark, N.J. 97101.

INFORMATION ON HEART SOUNDS AND PULSES:

Certain conditions may change the normal heart sounds. For example, the second sound (the "dub" in the "lub, dub") is often loud in hypertension because the increased arterial pressure causes the valves to close with greater force.

When the heart becomes weak from any cause (severe atherosclerosis, commonly)

the muscle contraction lacks force and the heart sounds often become "weak," "poor" or "distant" because the valves do not close with as much force.

Pulses:

1. Both the rate and strength of pulses can indicate various conditionsof the body. At rest, carotid arteries carry to the brain about 14 per cent of the total cardiac output. The carotid pulse is accordingly a large, strong pulse. The skeletal muscles in total receive about 10 per cent of the cardiac output; the radial, ulnar and posterior tibial pulses are accordingly each smaller pulses than the carotid. (The posterior tibial pulse is near the inner ankle.) A person with atherosclerosis of the leg arteries would have a weak to absent posterior tibial pulse. (This would also be true of the dorsalis pedis--the other major artery carrying blood to the foot.)

2. Arteriolar pulses can be used to monitor patients. Earlobe clips are often placed on patients in intensive-care units to detect the arteriolar pulse. If the patient were to go into shock, the ECG could remain unchanged, but the arteriolar pulse would stop.

Normal Heart Sounds and Murmurs:

1. To understand heart murmurs, it is important to understand the normal heartbeat. The following is a brief summary of the events that take place in the left ventricle. (Events in the right ventricle are comparable.)

a. When the left ventricle has filled with blood, it starts to contract. This puts pressure on the blood that is in it, which makes the blood press against the left A-V valve. This closes the valve because of the way the valve is shaped (see arrows in Figure 1A). The closing initiates the first heart sound that is heard through a stethoscope.

b. As contraction proceeds, it finally exerts enough pressure on blood in the ventricle for the blood to force open the aortic valve (see arrows in Figure 1B). Blood shoots into the aorta.

c. Next, the ventricle stops contracting and relaxes. The aorta, because it is elastic and has been streched by the blood the ventricle has squeezed into it, squeezes the blood back against the aortic valve, which is shaped so that it now closes (see Figure 1C). This initiates the second heart sound. (The aorta also squeezes blood forward into the arteries to supply the tissues.)

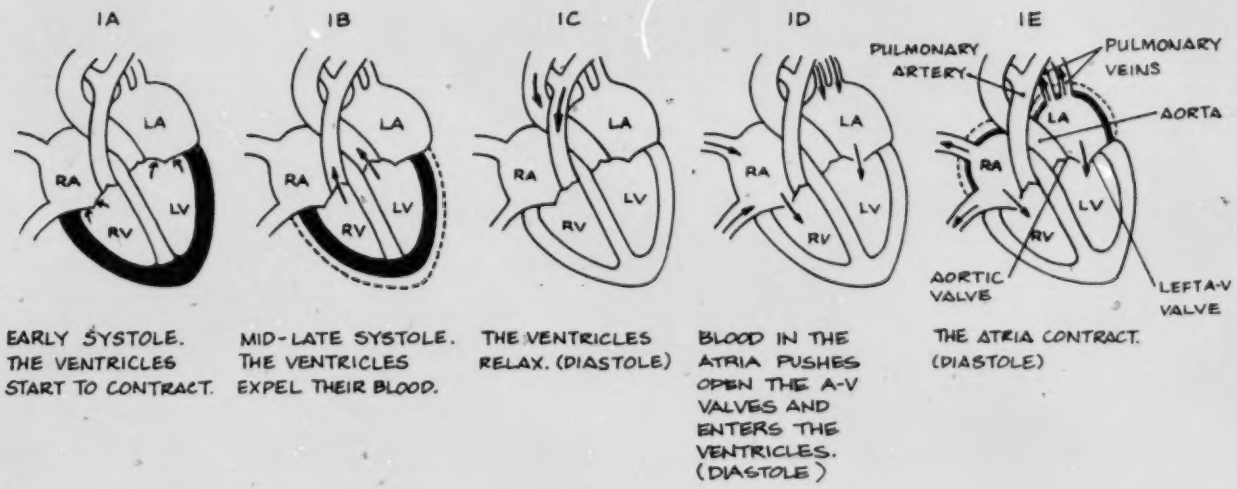


FIGURE 1: The heart cycle. Black, heavy lines indicate contraction.

d. The ventricle finally relaxes enough so that the pressure in it falls below the pressure in the left atrium. At that point blood in the atrium forces open the left A-V valve and fills up the left ventricle (see Figure 1D on preceeding page). Contraction of the atria squeezes a little more blood into the ventricles and completes diastole (Figure 1E on preceeding page).

2. It is the difference in pressure on the two sides of a valve that determines whether the valve is open or closed. For example, when the ventricles are relaxed during diastole, the pressure in them is lower than the pressure in the atria and in the aorta and pulmonary artery. As a result, the A-V valves are open and the semilunar valves are closed. The situation during systole is exactly the opposite.

3. A liquid flowing through a tube makes a sound only when it strikes the walls of the tube to make them vibrate, and this happens only when the flow is turbulent. Turbulence is an erratic change in the direction or speed of flow. It is the opposite of streamline flow, which is always parallel to the walls of the tube and silent. When a murmur occurs, it means there is turbulence where there normally is not.

Turbulence occurs when blood strikes something in its path, such as heart valves when it closes them. Turbulence also occurs, for other reasons, when the speed of a liquid's flow becomes too great. This happens when a valve is too narrow, for when a flowing liquid passes through a narrow passage, its pressure decreases and its speed increases.

Valve Defects:

1. The technical term for narrowing is "stenosis." Today, the most common cause of aortic stenosis is rheumatic fever, which is an autoimmune disease, i.e., one in which the body's own defense system destroys the valve. (Immunity will be discussed in Lessons 14 and 15.) Previous sensitization by contact with a certain streptococcus is needed for the disease to occur.

2. When a valve doesn't close completely because it has been distorted by scars, it is said to be "incompetent;" and backflow occurs through it. (Backflow is also called "regurgitation.") When the aortic valve is incompetent, some blood backflows from the aorta into the left ventricle.

A valve can be both stenotic and incompetent. This happens if it is so stiff from scarring that it can neither open nor close completely--it is more or less "stuck" in an intermediate position.

3. The left A-V valve is affected by rheumatic fever even more often than the aortic valve. When the left A-V valve is narrowed, the blood enters the left ventricle with greater speed, causing turbulence and a murmur.

The narrowing also causes a pressure buildup in the atrium, which is borne back through the blood in the pulmonary veins to the lung's capillaries, where it forces fluid to leave the capillaries through their walls. The fluid fills the alveoli and hinders oxygen absorption, so heavy breathing and fatigue occur in advanced rheumatic fever.

4. If a valvular defect is mild, a patient may show no ill effects; but if it is serious, the extra work the left ventricle must do can eventually, often after many years, cause irreversible damage to the ventricle. Before this happens, artificial valves can be used to replace the defective ones.

The most commonly used valve is the Starr-Edwards caged ball, shown in ST-5, first used in 1960. The ring portion is sewn around an appropriate-sized circular hole that is cut from the center of the diseased valve.

All parts of the valve are coated with an inert plastic, which minimizes the possibility of clots forming on the device. Over a period of a few months a layer of tissue grows over the ring portion of the valve.

There are also disc-type valves (Figure 2 on the following page) and tilting discs similar to a door on hinges, but these are less often used than the caged ball.

Around two-thirds to three-fourths of patients with artificial valves survive five years or more. In those who don't survive it is often because of heart-muscle damage that occurred prior to surgery. Also, the valves do obstruct forward flow somewhat; and clots can occur on them.

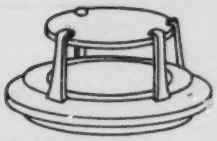


FIGURE 2: Disc valve.

5. During the valve-replacement operation, the heart is stopped and heart and lung function is taken over by a heart-lung machine, which pumps and oxygenates the blood and removes CO_2. The patient is also packed in ice to reduce the need for oxygen. If the surgery is lengthy, it is also necessary to provide oxygen for the heart itself. This is usually done by supplying diluted blood to the coronary arteries. (Diluting the blood reduces the risk of clot formation.)

INFORMATION ON LABORATORY ACTIVITY 5:

TEACHING NOTES:

1. The purpose of this activity is to give students the opportunity to perform several important clinical tests of heart function, including the arterial pulse, arteriolar pulse, heart sounds, and blood pressure.

2. Anticipated time: two to three periods.

3. Students should wear clothing appropriate to the study of heart and body sounds: clothing that need not be removed. You may wish to provide a separate room for females to use in Station I: Heart Sounds, to insure greater privacy.

4. The activities have been organized into five different stations in order to minimize equipment needs. Students can start at any station and move to any other available station until they have visited all. Some of the stations require two or more students working together.

5. The activity may be shortened by eliminating one or more stations.

6. At Station IV, students should be warned to wipe off the petroleum jelly (used in the first method of observing arteriolar pulse) from the finger before placing that finger on the BIP photocell.

7. Note that students will be recording answers to procedure questions out of numerical order because of the station arrangement.

8. You may wish to have students read ST-6 on blood pressure, before they perform Station II: Blood Pressure.

9. The school nurse or a local clinic or community hospital might lend a stethoscope or sphygmomanometer.

10. If your class has only one stethoscope, you may wish to have students take blood pressures by feeling for the pulse only.

If there are extra stethoscopes, each can be used either to set up a second Station I or II, or for the optional procedure given below.

11. If you have the necessary materials, you could make a teacher-student instrument to allow both you and a student to listen to the same heart at the same time. Use two stethoscopes which can be fairly easily disassembled and reassembled, and combine them as follows. Remove the bells from both stethoscopes. Connect the two open tubes to a Y-tube. Use rubber tubing to connect one bell to the Y-tube.

12. In females, the listening site over the tip of the heart may be covered by the breast or lower part of the bra. In such cases the closest accessible site will serve.

13. The students may well hear murmurs in some of their classmates. There is

a common murmur heard in many young people. It is rather soft, but is loudest in the semilunar valve area, which is its origin. (See LA-5, Figure 3). It is a murmur caused by blood as it enters the pulmonary artery. If such murmurs are found, explain that there is no cause for alarm. They are present because the high velocity with which blood is pumped out by vigorous young hearts, causes turbulence.

14. In taking blood pressure, the sounds usually disappear completely by 60 to 85 mm Hg. If the stethoscope is pressed too hard against the skin, however, the sounds may not disappear until the pressure drops below 60, because the stethoscope itself is compressing the artery.

15. At the end of the Information on LA-5 is an optional activity on listening to breathing, swallowing and stomach sounds, which may be reproduced and distributed.

MATERIALS: (for one of each station)

2 stethoscopes

cotton and alcohol (or alcohol swabs--enough to clean stethoscope earplugs between each use)

sphgmomanometer

bathroom scale

3 clocks or watches with second hands (or one visible throughout the room)

mm ruler

glass slide

petroleum jelly

30 sheets graph paper

ring stand and 2 clamps

hand lens (optional)

small lamp (optional)

Materials for Station IV, Arteriolar Pulse Rate by Photocell:

BIP

resistor, 1000Ω, 1/4 watt

resistor, 47KΩ, 1/4 watt

capacitor, electrolytic, 4.7-5.6 mfd

small piece plastic wrap

tape

photocell-Vactec VT 721 or similar

light bulb, #40 (6.3 V, 150 mA, miniature)

Tygon tubing, 5/16 inch I.D.

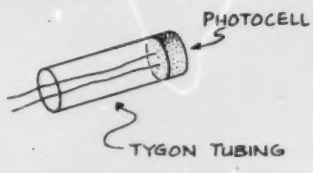
miniature light bulb receptacle (optional)

PREPARATION OF MATERIALS:

The circuitry for Station IV, Arteriolar Pulse Rate by Photocell, may be assembled as follows. (Note that the photocell and the #40 light bulb are supplied as standard accessory items with the BIP. If all the supplied photocells were used to make oximeters, you may wish to purchase two or three new ones. All of the wire described in the following instructions is the 24-gauge, insulated connecting wire supplied with the BIP. Note that soldering the connections is not necessary, although it does insure a better electrical connection.)

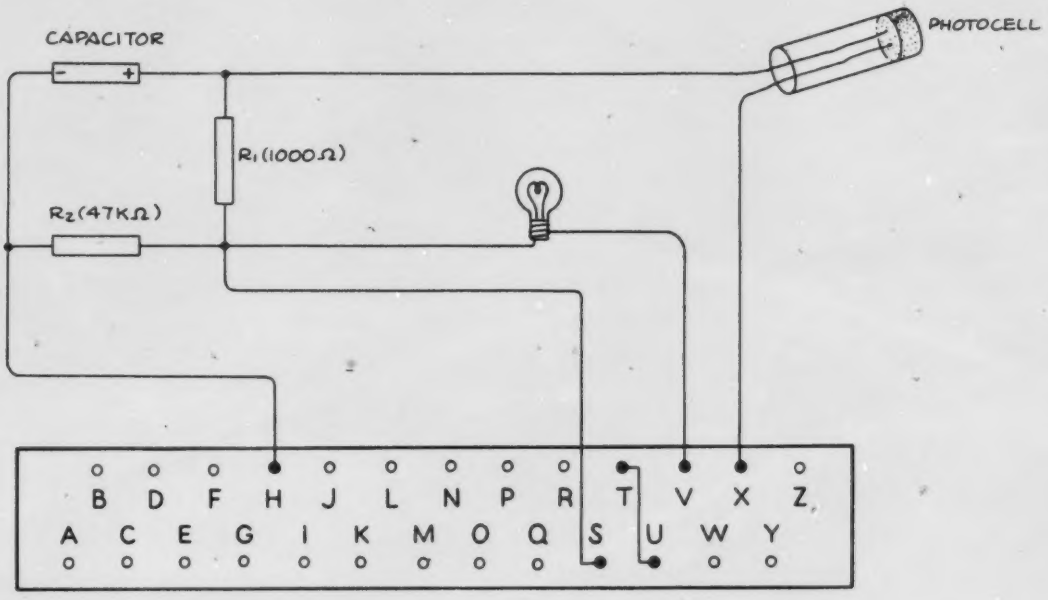
1. To mount the photocell, cut a 1-inch length of Tygon tubing, 5/16 inch I.D. Obtain two lengths of wire, each about 18 inches long. Strip 1/2 inch of insulation from both ends of each wire. To each lead from the photocell, connect by twisting together (or soldering) one of these wires. Mount the photocell in the tubing, making sure it is flush with one end (see figure). Put a piece of clear plastic wrap, such as Saranwrap, over the top of the photocell and fasten its sides down by taping it to the tubing. Avoid getting any tape over the top of the photocell, since this would interfere with light transmission. Clamp the photocell assembly to a ring stand as shown in the Student Procedure. (The face of the photocell should point upward.)

2. Obtain two lengths of wire, each about two feet long. (Only one is needed if a wire is already soldered to the tip of the bulb.) Strip one inch of insulation from an end of one of the wires. Wrap this end of the wire around the threads of the #40 miniature light bulb and fasten with tape. Strip 1/4 inch of insulation from the other end of this wire, and from both ends of the remaining wire. Solder one end of



the remaining wire to the tip of the light bulb. (Note: these wires may be connected to the terminals of a miniature light bulb receptacle, if one is available.) Clamp the light bulb to the ring stand as shown in the Student Procedure.

3. Connect the two resistors and the capacitor together as shown in the wiring diagram by twisting the leads together. If possible, solder the connections and trim the excess leads from the solder joint. Make sure that the positive lead of the capacitor is connected to R_1(1000Ω) and that the negative lead is connected to R_2(47KΩ). (The body of the capacitor should be marked with "+" and/or "-" signs.)



4. To the two resistor leads, connect a two-foot length of wire by wrapping or soldering. To this same joint, also connect one of the leads from the light bulb.

5. To the lead which joins R_1 and the capacitor, connect one of the leads from the photocell by wrapping or soldering the wire around the joint.

6. To the lead which joins R_2 and the capacitor, connect a two-foot length of wire.

7. Make the connections to the BIP programming panel as shown in the wiring diagram.

8. Adjust the variable voltage control (#3 in BIP Manual) until the bulb is very near full intensity.

9. If difficulties are experienced, note the following points.

a. Make sure the unit has been assembled properly. Are all connections intact?

b. Recheck programming.

c. Is variable voltage control adjusted properly?

d. Make sure the mA dial is set as close as possible to the null point.

e. When the finger is inserted, pressure should be exerted on it by both the bulb and the photocell. The pressure should be great enough for the subject to feel the pulse in the tip of the finger.

ANTICIPATED RESULTS:

There is no statement of anticipated results for Stations I, IIIC and V, since they are included in the Answers to Procedure Questions below.

Station II:

4. Systolic pressure is normally 100 to 130 mm Hg.

5. Diastolic pressure is normally 60 to 80 mm Hg.

Station IIIA: The Radial Pulse

7. a. The resting pulse is normally 55 to 95 beats per minute.

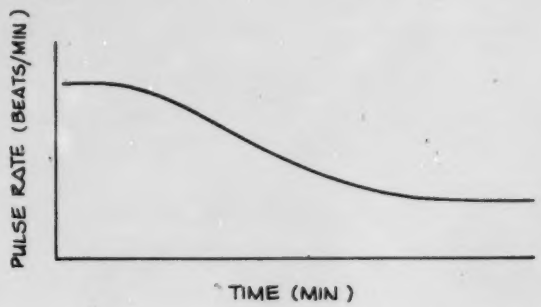
b. Immediately after standing it increases 10 to 20%.

c. By one minute after standing, it is back near the resting rate.

d. Exercise increases the pulse rate to 1.5 to 3 times the original rate.

e. The pulse rate decreases every minute after exercising has stopped, until it levels off at the resting rate of 55 to 95.

9. See graph.



Station IIIB:

Within seconds, part of the hand will flush. The areas that flush differ from person to person. Either artery will flush the entire hand in time, because the two systems are interconnected.

Station IV:

The arteriolar pulse rate is the same as the arterial pulse rate.

ANSWERS TO PROCEDURE QUESTIONS:

1. The tip of the heart.

2. Sounds are usually lower in pitch at the tip and longer in duration.

3. The pulse rate increases immediately upon standing. After one minute it is close to the resting pulse again.

4. The pulse rate during exercise should be 1.5 to three times the resting rate.

5. It decreased toward resting level.

6. The increased pulse rate means increased blood flow. This meets the increased needs of working muscles for oxygen, glucose and removal of wastes.

7. The carotid pulse occurs just before the radial pulse.

8. The carotid artery is closer to the heart, so it expands first.

9. The needle jumps farther after exercise. This means more blood is being pumped by each heartbeat during exercise than before exercise.

ANSWERS TO DISCUSSION QUESTIONS:

1. When a fluid flows through a constriction, the flow becomes turbulent. The turbulence produces the noise.

2. The answers should vary widely. See Anticipated Results.

3. The answers will again vary. For the age group of 15 to 20 years, the normal range for systolic blood pressure is 115 ± 15 mm Hg and for diastolic blood pressure is 72.5 ± 12.5 mm Hg.

4. When the cuff pressure is above systolic blood pressure, the artery is closed and no blood gets through. The needle does not jump. When the cuff pressure is below systolic but above the diastolic blood pressure, the artery is suddenly forced open during each heartbeat by the pressure of blood against the arterial walls. This sudden opening of the artery registers as a jump of the needle with each heartbeat. When the cuff pressure is below diastolic blood pressure, the artery is open all the time. The needle does not jump.

BREATHING AND OTHER BODY SOUNDS:

Note: before beginning this activity, be sure you have read the information on the stethoscope described in the Procedure for Station I.

MATERIALS:

stethoscope	tape
soda crackers	scissors
drinking cup	mm ruler

PROCEDURE:

PART I: OUTLINING THE LUNG

1. Place the bell or diaphragm of the stethoscope on another student's back at a spot that is between the shoulder blades and about 4 cm to the left of the spine. Place a very small piece of tape here to mark the spot (Figure 1).

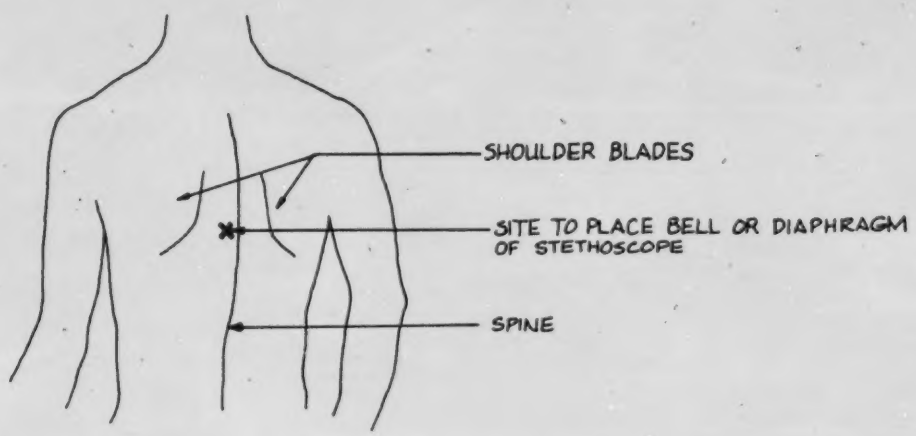


FIGURE 1

2. Have the subject breathe deeply through the mouth a few times, while you listen for the sound of air entering and leaving the left lung. Let the subject rest for a couple of minutes to avoid hyperventilation.

3. Once again have the subject breathe deeply through the mouth. During the deep breathing, move the bell or diaphragm of the stethoscope to new listening sites that are directly below the tape. Listen for the sound of air moving through the subject's lung. Continue listening in the area below the tape untl you can no longer hear air moving through the lung. Mark this area with another small piece of tape.

4. Put the stethoscope back on the last area where you could still hear air moving. Continue listening with the stethoscope moving toward the left. When you no longer hear air moving, place another small piece of tape. Continue listening, now moving upward toward the left shoulder, and marking areas where air sounds can no longer be heard. You should end up with an outline of your partner's left lung (Figure 2 on the following page).

5. Draw an outline, on a sheet of paper, of the subject's back and shoulders. Sketch in the approximate location of the lung.

How does the size and location of your left lung compare to that of the subject you tested? (1)

PART II: SOUNDS WHILE CHEWING AND SWALLOWING

1. Place the bell of the stethoscope on the V depression in the subject's neck (Figure 3 below).

2. Have the subject drink a few sips of water while you listen.

3. Have the subject chew a cracker and swallow it while you listen with the stethoscope.

Describe the sounds you hear during chewing. (2)

Describe the sounds you hear during swallowing. (3)

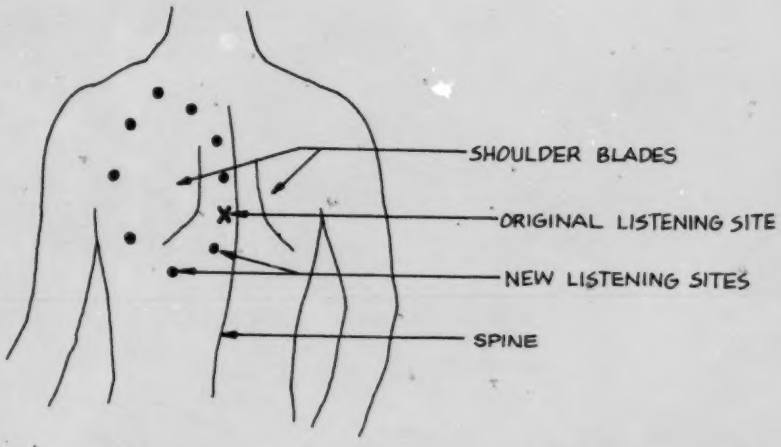


FIGURE 2: Using the stethoscope to outline a lung.

PART III: SOUNDS FROM THE STOMACH

1. Place the bell of the stethoscope on a spot over the subject's stomach region. Listen for a minute or two. Describe the sounds you hear from the stomach and/or intestines, how often they occur, and how long they last. (4)

2. Have the subject drink a few sips of water while you listen. Describe the noises heard. (5)

Speculate on the cause of stomach noises. (6)

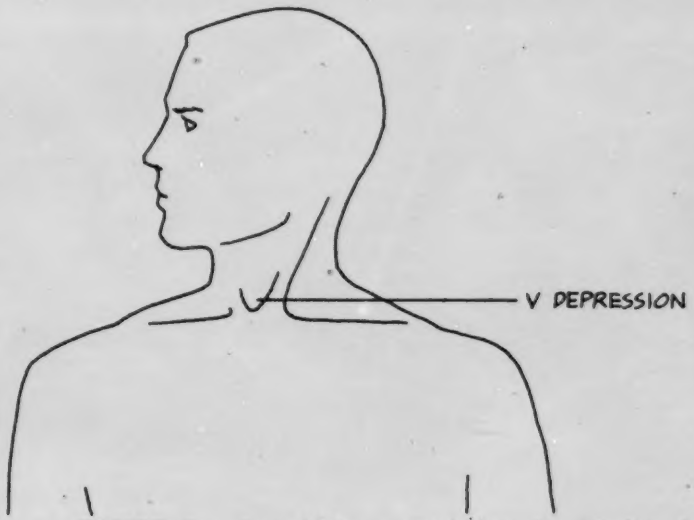


FIGURE 3: Locating a position to listen to chewing and swallowing sounds.

PART IV: SOUNDS FROM THE JOINTS

1. Remove clothing (roll up pants leg or sleeve) from the area to be monitored. Place the bell or diaphragm on the skin near the bending part of the knee or elbow Have the subject bend the joint while you listen for sounds. If someone has a "bad knee" or a joint that "squeaks" or "pops" often, listen to it.

Record the name of the joint, the side of the body, its condition (football injury, no sound, squeaky), and the sounds you hear. (7)

PART V: THE POSTERIOR TIBIAL PULSE (Pulse beat from the posterior tibial artery)

The pulse in the ankles is an important diagnostic test for atherosclerosis in the legs. An absent or even weak pulse in the ankle indicates an impairment of circulation in the leg.

1. Have the subject remove the shoe and sock from the right foot. (Females can leave on sheer hosiery.)

2. Next have the subject cross one leg over the other to elevate the bare foot from the floor.

3. Place your index and middle finger on the inner side of the subject's ankle at a point that is about midway between the ankle bone and the heel. Press fairly firmly to feel the pulse (Figure 4).

4. When you have located the pulse, count the number of beats during a 15-second interval. Record the number of beats, including the uncertainty.

5. Repeat Step 4 twice. Use these data to calculate the average pulse in beats per minute. Include the uncertainty.

6. Compare the pulse at several sites (such as radial, ulnar, carotid, posterior tibial) in terms of beats per minute, strength of beat, and duration of beat. Suggest reasons for any differences. (8)

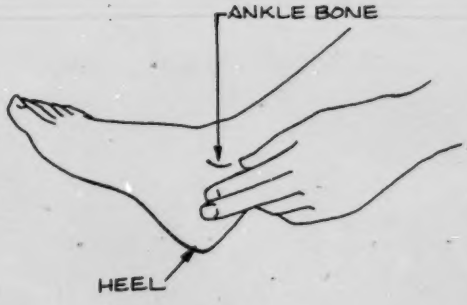


FIGURE 4: Locating the posterior tibial pulse.

ANSWERS TO PROCEDURE QUESTIONS:

1. Answers will vary. There is a normal variation in the size and shape of internal organs, just as with any external feature.

2. and 3. Answers will vary.

4. Gurgles, sometimes high-pitched. They may occur once every minute, or much more often, even constantly. They usually last at least 5 seconds, often longer.

5. The sounds of sipping and swallowing made by the mouth and throat can be heard. The stomach may produce gurgling sounds as well.

6. Stomach noises are originated by peristalsis.

7. Answers will vary.

8. The pulse rate should be the same at all sites. The strength of the pulse depends upon distance from the heart and the diameter of the artery. Any differences noted in duration should be insignificant.

LESSON 6: THE IMPORTANCE OF BLOOD PRESSURE

RATIONALE:

One of the most vital tests of circulatory function is arterial blood pressure. Adequate blood pressure is necessary for adequate blood flow. On the other hand, blood pressure that is too high (hypertension) is a serious health problem in the U.S.; and screening people for it is an important task of preventive medicine. ST-6 provides information on both normal and abnormal blood pressure. The laboratory activity, which is a continuation of LA-5, includes a test of blood pressure.

OBJECTIVES:

The student will:

- define the terms systole, diastole, systolic blood pressure, diastolic blood pressure.
- state which number in a blood pressure reading represents systolic and which diastolic pressure.
- state the desirable range of systolic and diastolic blood pressures in people between 15 and 20 years of age, and between 45 and 50 years of age.

·state why low blood pressure is dangerous.

·state why hypertension is dangerous.

·state the general relation between age and blood pressure.

·state at least one factor that lowers and raises the blood pressure.

SEQUENCE: LA-5 (continued); ST-6

SUGGESTIONS:

1. You may wish to provide a rough analogy in order to explain one way to treat hypertension. Just as air can be let out of a tire to reduce air pressure, the volume of blood can be decreased to reduce blood pressure. This is done with diuretics (drugs that increase urine formation). Loss of urine in turn reduces the volume of fluids in the body, including blood. Such drugs include furosemide and chlorothiazide. Reducing sodium intake in the diet also reduces blood volume as was discussed in the Nutrition Unit.

Another analogy may be useful to demonstrate how hypertension may be treated. If one could somehow make the tire larger while keeping the same amount of air inside, the air pressure would drop. This is analogous to dilating the arterioles and small arteries in the body. Drugs are used to dilate these vessels by relaxing the muscle cells in the vessel walls. Such drugs (vasodilators) include reserpine and methyldopa.

2. Pressure was treated in the Respiration Unit, Lesson 13, and in this unit, ST-2. As a review, a bicycle inner tube and a bicyle pump may be used to demonstrate the pressure-volume relationship. (Blowing up a balloon will also serve the same purpose.) The more air pumped in, the higher the air pressure. Students will find that it is harder to indent the tire or balloon with a finger when there is more air inside. This is because the air inside is pushing harder against the wall. Futhermore, one has to push harder with the pump when the pressure in the tire is high. This is analogous to the heart working harder in cases of hypertension.

Of course, arteries differ from a tire or balloon in that blood continuously exits from them into arterioles. This tends to lower the blood pressure, but never completely, because the heart keeps pumping blood in.

It is important to note that this analogy applies mainly to arterial blood pressure. Arterial hypertension is the common form of hypertension. Venous hypertension is less common and usually occurs after prolonged pre-existing heart disease.

3. You may wish to have students measure blood pressure before and immediately after performing strenuous exercise--25 jumping jacks, running in place for 30 seconds, or stepping up and down (using the step-test pattern) quickly for 30 seconds. Systolic blood pressure rises during exercise. Blood pressure may be measured under other conditions too, such as immediately after standing up from a reclining position.

4. You may wish to discuss the danger that hypertension can cause an aneurism to burst, or the role of pressure monitors in maintaining the blood pressure (Information below).

5. The students might be interested in speculating on appropriate emergency treatment for individuals suffering from dangerously low blood pressure for the following reasons.

a. Blood loss (add blood and, of course, stop the loss if possible)

b. Water loss (add fluid to the blood, e.g., saline solution adjusted to the proper osmotic pressure)

c. Arteriolar dilation (give drugs that will constrict the arterioles, i.e., vasoconstrictors)

6. Ask students to conjecture on the technical term for low blood pressure (hypotension).

7. a. Ask students to bring pills from home which contain drugs needed for LA-7. Examples are "Vivarin," "Sominex," "Com-poz," "Allerest," diet pills, sleeping pills, etc.

b. Be sure a culture of Daphnia and a supply of aquarium or aged tap water is available for LA-7. Cultures should be ordered so as to arrive about one day prior to the activity.

INFORMATION ON BLOOD PRESSURE:

1. Blood pressure is monitored by certain nerve cells (monitors) located in the walls of the carotid arteries and the aorta. (The carotid arteries course through the neck and carry blood to the brain.) If the walls of these vessels are stretched because of a rise in blood pressure, the monitors send signals to the brain. The brain then sends signals through nerves to the heart to slow down its rate, and through nerves to the arterioles to dilate. These effects help reduce blood pressure to normal. In people with hypertension, this system may be faulty in some way.

Conversely, when blood pressure drops, the nerve monitors send different signals to the brain. The brain responds by acting to raise the blood pressure through raising the heart rate and constricting arterioles. This happens for example when someone stands up quickly from a lying or sitting position. In this situation blood drains out of the head and neck. The loss of blood from the neck region lowers the blood pressure in the carotid arteries. Monitors sense the drop in pressure and act to raise it. The pressure of blood flowing to the brain is thus quickly restored to a normal level.

2. Atherosclerosis ("hardening" of the arteries) was considered in Unit II and will be presented in detail in Lesson 9. The popular idea that atherosclerosis causes hypertension is not true. The two often do occur together, however.

3. In addition to increasing the risk of a heart attack, hypertension is also dangerous when an aneurism is present. An aneurism is a weak area that balloons out from a vessel wall. It is usually caused by atherosclerosis. Hypertension may aggravate the ballooning and thus make the vessel more likely to burst. Burst aneurism is a common cause of stroke when it occurs in arteries in the brain.

4. In general, factors which increase the heart rate also increase the blood pressure. Thus the two usually go up during exercise and excitement. It is possible, however, for heart rate and blood pressure to be affected differently by the same event. For example, at a high external temperature, heart rate may increase but blood pressure may remain the same or even drop. The blood pressure effect is complex. First the high environmental temperature causes arterioles in the skin to dilate and the blood flow near the skin to increase. (This process helps to cool the body--the blood loses heat to the environment.) As a result of the dilation of the arterioles, the blood flows out of the arteries more rapidly, and the arteries may end up with a reduced volume of blood leading to a drop in blood pressure.

LESSON 7: FACTORS THAT REGULATE THE CARDIAC OUTPUT

RATIONALE:

This lesson presents the ways in which the cardiac output is varied to meet the body's changing needs. The cardiac output depends on two things: the heart rate and the stroke volume. ST-7 shows how these two factors are regulated. LA-7 gives students the opportunity to test the effects of several factors upon the heart rate of Daphnia.

OBJECTIVES:

The student will:

- state the effects of the vagus nerve and the "accelerator" nerve upon heart rate.

- define the terms stroke volume and cardiac output.
- state three conditions that increase the cardiac output and two conditions that decrease it with respect to the resting state.
- state two changes in the action of the heart which enable the heart to increase its output during exercise.
- state why more blood returns to the heart during exercise than during rest.
- describe the effects of temperature and of specific drugs and chemicals on the heart rate of Daphnia.

SEQUENCE: ST-7; LA-7

SUGGESTIONS:

1. Various conditions that affect the heart rate were presented in ST-5. You may wish to review them and have students speculate on whether more or fewer signals travel down (1) the vagus and (2) the "accelerator" nerve in each case. Students may also speculate on whether epinephrin (adrenalin) is secreted in each case. Epinephrin is secreted during excitement and exercise.

2. You may wish to present, if you have not already done so, the role of the blood-pressure monitors in bringing about a change in heart rate. Briefly, the monitors are located in arteries, and send signals to the brain regarding blood pressure. The brain responds to these signals through the vagus and "accelerator" nerves. Details on this system are in Lesson 6, Information on Blood Pressure, and in Information on Cardiac Output, below.

3. You may wish to show students the formulas for norepinephrine (Figure 1), acetylcholine (Figure 2), and epinephrin (Figure 3) and have them identify functional groups such as alcohols (-OH) and amines ($-NH_2$). All three compounds are synthesized in the body from amino acids.

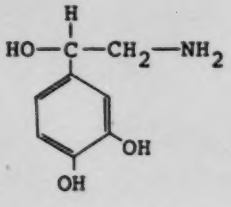


FIGURE 1
Norepinephrine.

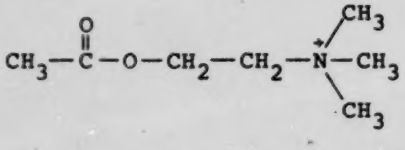


FIGURE 2
Acetylcholine.

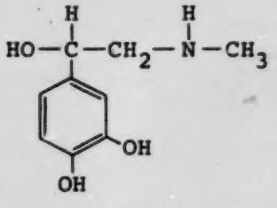


FIGURE 3
Epinephrin.

Note that the nitrogen in acetylcholine (Figure 2) appears different than in the nitrogen compounds the students have seen thus far. Perhaps the simplest way to explain the nitrogen configuration in acetylcholine is to point out that it is equivalent to the configuration in NH_4^+. Ammonia has a tetrahedral shape (Figure 4) with an unshared pair of electrons at one vertex of the tetrahedron. If a H^+ ion is added, the hydrogen atom will share those two electrons with the nitrogen atom to make a fourth bond. This results in a net charge of +1 on the molecule. In acetylcholine the fourth bond is to CH_3 rather than H.

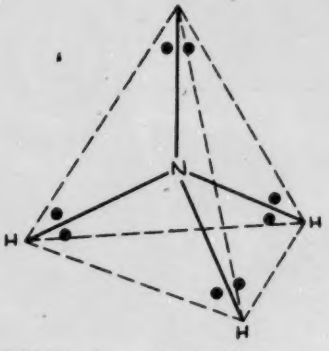


FIGURE 4: Ammonia.

INFORMATION ON CARDIAC OUTPUT:

Under certain conditions the distribution of blood to different organs changes. For example, during exercise more blood flows through skeletal muscles than during rest. This happens when the arterioles in muscles dilate. Dilation of arterioles in muscles occurs during

exercise as a result of at least two factors. (1) An area in the brain called the vasomotor center sends signals through nerves to the arterioles in skeletal muscle. These signals make the arterioles dilate. (2) When skeletal muscles work, the accumulation of CO_2 and the decrease in oxygen in them both cause the dilation of arterioles, through directly affecting the smooth muscle cells in the walls of the arterioles.

When arterioles dilate, blood leaves the arteries much faster than it does at rest. This would drop the blood pressure in the arteries to dangerously low levels if the heart did not compensate by pumping more blood into the arteries, i.e., by raising the cardiac output. The heart increases its cardiac output by (1) increasing its stroke volume (the volume of blood ejected per beat) and (2) increasing the heart rate.

The stroke volume of the heart is increased when the heart contracts harder during each beat. During exercise the heart contracts harder for two reasons. First, there is the effect of the hormone epinephrin (adrenalin). Second, the "accelerator" nerve, which increases the heart rate, also makes the heart contract harder. It is interesting to note that one of the commonest drugs used for heart disease, digitalis, works by increasing the force of the heart's contraction.

The heart rate also rises during exercise for two reasons. First the "accelerator" and vagus nerves act to raise the heart rate. Second, there is the effect of epinephrin. The nerves and epinephrin increase the heart rate by affecting the portion of the heart called the sinoatrial node, a mass of cells in the right atrium. The sinoatrial node is responsible for determining the heart rate. The nerves act by releasing certain chemicals into the sinoatrial node, while epinephrin reaches the node via the blood. The sinoatrial node will be discussed in Lesson 8. Since heart rate is controlled by a small portion of the heart, the nerves and epinephrin need only affect that small region to change the heart rate. In contrast, the force of contraction is a property of the entire heart muscle. The factors that make the heart contract harder, work by directly affecting the biochemical events of contraction which take place inside heart muscle cells.

The nerves that go to the heart come from two centers located in the medulla of the brain, the cardioinhibitory (CI) center and the vasomotor center. The CI center sends impulses down the vagus nerve to the heart which slows its rate; the more impulses, the slower the heart rate. In contrast, the vasomotor center sends impulses down the "accelerator" nerve to the heart which speeds the heart rate. These different effects upon heart rate occur because the "accelerator" nerve releases from its nerve endings the compound norepinephrine (Figure 1) into the heart, while the vagus nerve releases acetylcholine (Figure 2). Through their different effects on the sinoatrial node, these two chemicals have opposite effects on the heart rate.

The hormone epinephrin (Figure 3), also called adrenalin, is secreted by the adrenal glands. This happens during exercise when the vasomotor center sends signals down nerves that stimulate these glands to secrete. Epinephrin is chemically and physiologically like norepinephrine, but the latter is secreted only at nerve endings. Like norepinephrine, epinephrin both speeds up the heart rate and causes the heart to contract more forcefully.

The heart could not increase its output unless it received more blood to pump out. During exercise, more blood returns to the heart from the veins. The vasomotor center is stimulated by the excitement of exercise, as well as by the decrease in blood oxygen and the increase in blood CO_2. The vasomotor center then responds to these stimuli by sending impulses to the veins which cause the veins to constrict and therefore empty more of their blood into the heart. This augments the greater venous blood return which already comes from moving the limbs and breathing harder.

In Lesson 6 the role of blood pressure monitors was presented. It was stated that when blood pressure falls, the monitors send signals to the brain, which responds by raising the heart rate and constricting arterioles. This restores the blood pressure. The parts of the brain referred to are the vasomotor and CI centers. During exercise, the pressure monitors may play only a minor role in raising the heart rate to maintain blood pressure. It is thought that the changes in cardiac function during exercise described above are largely in response to signals from the brain that are generated whenever the signals to move muscles are given.

INFORMATION ON LABORATORY ACTIVITY 7:

TEACHING NOTES:

1. The purpose of this activity is to determine how temperature and some common drugs affect the heart rate in Daphnia.

2. Anticipated time: one to two periods.

3. Daphnia can be obtained from ponds or lakes during most of the year, from a biological supply house, or sometimes from aquarium supply stores. Daphnia magna is the largest and therefore the most desirable species for this activity. D. pulex and D. longispina are smaller but satisfactory. If Daphnia are collected locally, culture them in the water in which they are found. If they are not found locally, use pond water or water from an established and well-balanced aquarium. The culture should be aerated, kept at a temperature of 20 to 25 °C, and kept out of direct sunlight.

4. If the culture is kept for more than two weeks the organisms can starve. If the Daphnia are to be stored, feed them a yeast suspension of $\frac{1}{4}$ package of dry "active" yeast in 250 ml of pond or distilled water. Add just enough of the yeast suspension to turn the water in the culture milky. Whenever the water becomes clear, add more yeast suspension. After the activity is completed, the leftover Daphnia could be provided to students who want to do more research on the effect of drugs on heart rate.

5. The diaphragm setting on the microscope should provide the least amount of light that enables one to see the heart clearly.

6. Instead of using an ice-water bath, students may place the slide on an ice cube for a few minutes. This method is simpler, but does not permit the temperature to be measured.

7. The students should be cautioned about using too much petroleum jelly since it may cover the Daphnia, making them hard to see. It may also come in contact with the microscope lens.

MATERIALS: (for 10 set-ups)

culture of live Daphnia
ice cubes, about 5 kg
10 water baths
petroleum jelly
lens paper
10 depression slides, depression 18 mm diam. x 0.8 mm deep (Carolina 63-2200)
20 medicine droppers
10 microscopes with light source
10 to 20 toothpicks
dropper bottle each of solutions of alcohol, nicotine, and aspirin
samples brought in by students
500 ml distilled or pond water
clock with second hand
10 thermometers
20 beakers, 150- or 250-ml
20 beakers, 50-ml
10 graduated cylinders, 10-ml
10 stirring rods
10 mortars and pestles (or other devices for crushing)

PREPARATION OF REAGENTS:

Alcohol Solution: Add sufficient water (distilled, pond or aquarium) to 5 ml of 95% ethyl alcohol to make 100 ml.

Nicotine Solution: Add the tobacco from five cigarettes (minus the paper and filter) to 25 ± 5 ml water (distilled, pond or aquarium).

Aspirin Solution: Crush one tablet in 10.0 ml of water (distilled, pond or aquarium).

The directions call for water in the preparations to be either distilled, pond

or aquarium. You may also use "aged" tap water. Filtering of the solutions is not necessary. If any of the solutions kills several Daphnia, dilute slightly.

The nicotine and aspirin solutions may be made up the day before the activity or just prior to class. A beaker may be more convenient than a dropper bottle for the stock solution of nicotine, because the tobacco tends to clog the dropper.

ANTICIPATED RESULTS:

Determinations in our laboratory using several Daphnia resulted in the following data for heart rate. Each letter represents a different Daphnia, and all heart rates are in beats per minute.

CONTROL

Daphnia	Heart rate before 3 drops of pond water added	Heart rate at the time after 3 drops pond water added	
		< 30 seconds	2-3 min.
A	300	292	268
B	260	292	244
C	304	308	268

DRUGS

Drug	Daphnia	Heart rate before drug added	Heart rate at the time after drug added	
			< 30 seconds	2-3min.
Alcohol: (95% ethanol)	D	272	224	168
	E	312	232	188
Nicotine: (Kent king-size cigarettes)	F	316	256	192
	G	260	260	208
	H	288	256	208
Aspirin: (Bayer, 1 tablet ≈ 0.3g)	I	268	260	252
	J	316	280	296
	K	336	332	312

ANSWERS TO DISCUSSION QUESTIONS:

1. At the concentrations used, both the alcohol and the nicotine solutions depressed the heart rate. The changes observed with the aspirin solution are probably not significant.

2. The answers may vary. See also the answer to Question 3.

3. a. Drugs vary in terms of their solubility in water. (For example, only about 10% of the aspirin went into solution.)

b. Some drugs may take longer to act than others.

c. There may be significant individual variation in the response of Daphnia to various drugs.

d. Some the Daphnia may have been injured during the procedure, which could be expected to have an effect on heart rate.

4. The drugs may have a long-lasting effect, which could obscure the effect of any new drug applied.

5. Heart rate increases with temperature in the range tested.

6. Because of the great difference between Daphnia and humans, tests on this organism may not be valid indicators of effects on humans. It might be pointed out, however, that the results obtained with alcohol and nicotine are generally consistent with the effects of these drugs on humans. The effects of oral ingestion of nicotine (as opposed to the slight amounts absorbed from the inhalation of tobacco smoke) include a slowed heart rate, vomiting, disturbed vision and often death. Nicotine is a highly toxic drug, acting with a rapidity comparable to that of cyanide.

LESSON 8: ELECTROCARDIOGRAPHY AND THE ELECTRICAL NATURE OF THE HEARTBEAT

RATIONALE:

One of the goals of this unit is to familiarize students with the medical instruments and techniques used in studying the heart and circulatory system. In previous lessons students have examined the heart's anatomy and performed various clinical tests on the heart. In ST-8 the electrical activity of the heart is related to the events of the cardiac cycle. This discussion of the cardiac cycle leads to a detailed description of the electrocardiograph showing normal and abnormal conditions. In LA-8 the students use the BIP to study the heart wave forms.

OBJECTIVES:

The student will:

- state the function of the sinoatrial node.
- define and correctly use the terms ECG, electrocardiogram and electrocardiograph.
- describe how at least one abnormality can change the ECG.
- identify and label on an electrocardiogram, the P, QRS and T waves.
- display and identify P, R and T waves of the cardiac waveform by means of the BIP diodes.
- identify the events of the cardiac cycle corresponding to the P, QRS and T waves.

SEQUENCE: ST-8; LA-8

SUGGESTIONS:

1. Note that the student copies of the Student Text for Units I and II will be needed in class during Activity 9.

2. You may wish to review the factors that change the heart rate (nerves and epinephrin). These operate by affecting the sinoatrial node.

3. It may be useful to begin this lesson by outlining the firing sequence of cardiac muscle as summarized below.

a. Electrical activity is initiated within the heart at the sinoatrial (SA) node. An electrical wave, called an activation wave, sweeps from this node through the heart. First it goes through the atria and to the atrioventricular node (Figure 1), then through the ventricles.

b. Contractions of cardiac muscle is triggered by the activation wave.

c. Following contraction the heart rests.

4. In reviewing this lesson it may be useful to emphasize the following.

a. As the activation wave passes through cardiac muscle, weak electrical potentials are transmitted to the surface of the body.

b. This electrical activity can be monitored, amplified and recorded, the recordings being very important since they reflect the condition of the heart.

c. Electrocardiography is the study of electrical activity of the heart.

d. The signals are amplified and then recorded on a tape and called an electrocardiogram.

e. The amplified impulses can be transferred to an oscilloscope and presented on the oscilloscope screen as a waveform.

f. The appearance of the waveform on the screen changes when the electrode positions are changed.

5. In ST-8 only one heart pacemaker is mentioned (the SA node). This was done for simplicity, but you may wish to point out that there is more to the story. The impulse from the SA node passes to the artioventricular (AV) node and then to the ventricules. (See Figure 1.) The AV node is also a pacemaker; and if the SA node is incapacitated, the AV node causes the heart to beat at a rate of about 45 to 60 times per minute. In Section 8-1, we pointed out that when the pacemaker impulse is blocked before reading the ventricles (for example, from a heart attack), then the "basic heart rhythm" takes over. This basic heart rhythm is due to the ventricles which can beat about 30 times a minute when the impulses from the SA and AV nodes do not reach the ventricles.

INFORMATION ON THE ELECTRICAL ACTIVITY OF THE HEART, AND ITS MEASUREMENT:

Origin of the heartbeat

The activation wave may be considered in terms of ion concentrations inside and outside the cell. Cells are characterized by a higher concentration of positive ions outside the cell than in the fluid inside the cell. This arises because the cell membrane has a greater tendency to allow positive ions to leak out than to enter.

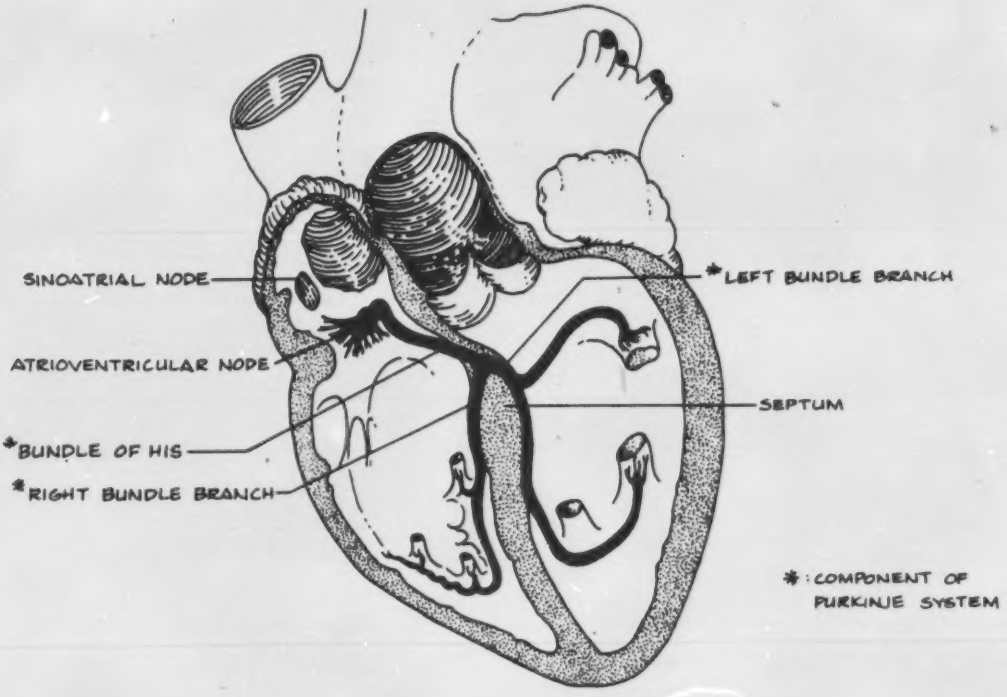


FIGURE 1: Conducting system of the heart.

When an electrical wave is carried through heart tissue, however, the positive ions that have collected outside rush into the cells, leaving the outside of the cells temporarily more negative than they were before, and making the inside temporarily more positive. This ionic movement first occurs at one end of the heart and

then occurs sequentially throughout it. The sequential spread of this ionic movement through the heart gives rise to an electrical current. It is this current that is the activation wave. As the current flows, the polarity on the two sides of the cell membrane is reversed; this electrical current or wave is therefore also called a depolarization wave. As the depolarization wave moves along the heart muscle cells, it causes the cells to contract. After contraction, a wave of repolarization restores the positivity outside the cells, readying the heart for the next depolarication wave.

The depolarization wave, also called the activation wave, is initiated from within the heart itself each time in a specialized bundle of cells called the sinoatrial (SA) node, located near the opening of the superior vena cava into the right atrium (Figure 1). From there it sweeps through the atria and reaches the atrioventricular node, which delays the wave about 0.08 second. Then the depolarization wave is conducted rapidly down the septum to the ventricles through a specialized conduction system called the Purkinje fiber system. This wave causes a corresponding contraction (systole).

The cells of the SA node and conduction system act like nerves in that they initiate and propagate impulses; microscopically, however, they resemble not nerves but heart muscle cells, from which they are distinguishable mainly by their larger diameter and greater glycogen content.

The Electrocardiogram

The electrical events of the cardiac cycle could be recorded by placing electrodes directly into the heart muscle. But since body fluids are highly conductive, current spreads out away from the heart and voltage differences can be recorded "at a distance" (see Figure 2). There is a sharp drop in voltage at the skin; of the 135 millivolts measured in the heart, only a few millivolts are measured at the skin surface where electrocardiographic electrodes are placed. In a typical ECG (Figure 3), each large unit in the vertical direction represents 1 millivolt and each large horizontal unit 0.2 second.

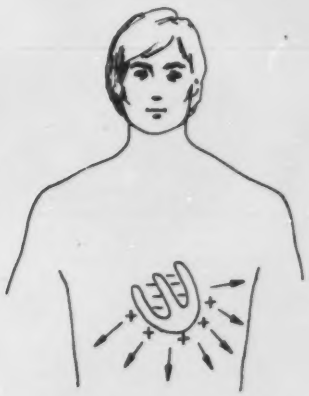


FIGURE 2: Current spreads throughout the body from the heart.

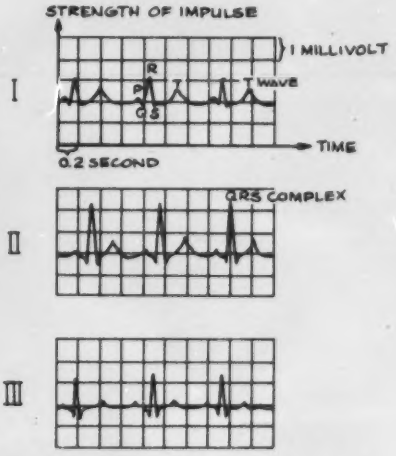


FIGURE 3: A typical electrocardiogram (ECG).

In Figure 4, (on the following page) a single arrow is used to represent the average direction in which the activation wave is moving at a given time during the cycle of the heart's activity. Figure 4 shows the depolarization wave, represented by the arrow, as it starts at the SA node and moves leftward and downward. Another way to say this is that Electrode B is the one toward which the activation wave is moving. An electrocardiograph connected to these electrodes records an upward deflection because the activation wave is more toward the anode, B, than toward A. The circuitry of the ECG is such that an upward deflection occurs. This is a general rule: when the heart's activation wave is moving more toward the positive than the

negative electrode, the ECG will show an upward deflection. Movement away from the positive electrode causes a downward deflection.

Notice in Figure 4 that the atrial activation wave also travels downward. Hence, a positive electrode placed on a leg would produce an upward deflection on the ECG because the wave is travelling toward the legs.

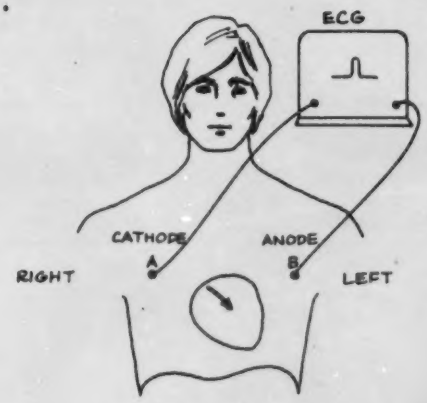


FIGURE 4: The atrial activation wave causes an upward deflection in the ECG.

In actual medical practice, electrodes are not placed on the chest at positions A and B shown in Figure 4. Three other pairs of electrode positions (or "leads") are used commonly and they are shown in Figure 5. In lead I the negative electrode is placed on the right forearm and the positive electrode on the left forearm. Lead II also involves placement of the negative electrode on the right forearm, but the positive electrode is placed on the left calf. Lead III, the other common positive electrode on the left calf (as in Lead II) and the negative on the left forearm. The ECG's for the three positions differ (Figure 5). The

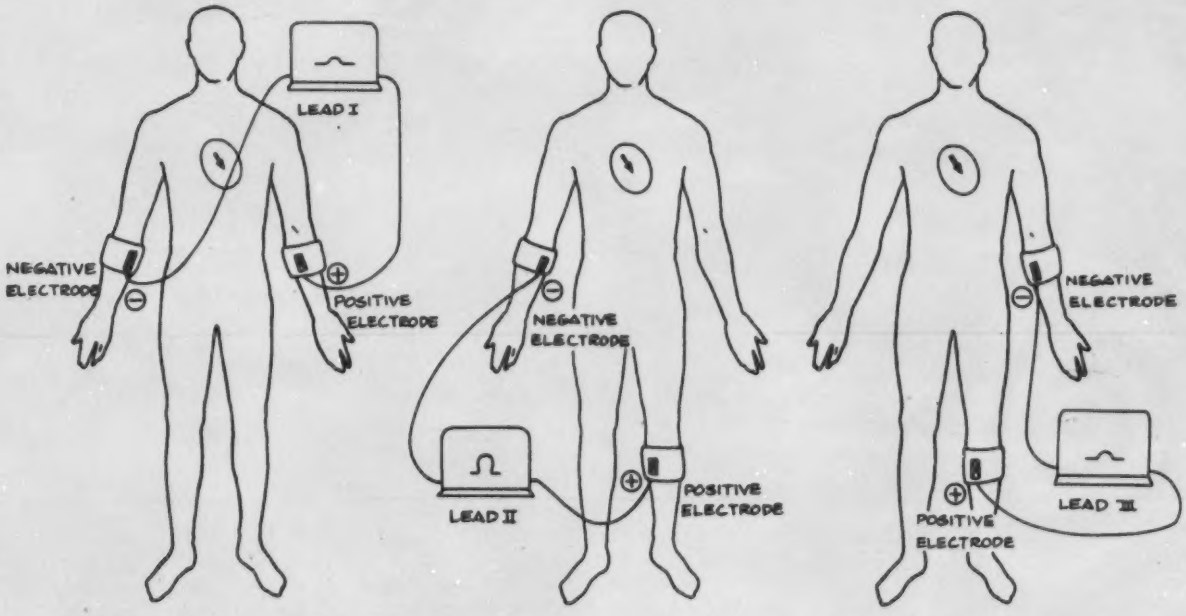


FIGURE 5: Three different conventional arrangements of electrodes for recording the ECG. The arrow in the heart represents the direction of the atrial activation wave; the P wave it produces is shown for each lead.

differences can also be seen in Figure 3; the Roman numerals represent the three lead positions. More than one lead is used because it is possible for one lead to produce a normal waveform, while another detects an abnormality.

In medical practice, the activity detected by one lead position at a time is recorded, first I, then II, then III. The atrial activation wave is called the P wave corresponding to each lead psoition may be seen in Figure 5. Note that in all three positions, the P wave involves an upward deflection. The upward direction results, because the arrow representing the atrial activation wave is pointing genrrally toward the positive electrode of each lead.

The ECG pattern

The atrial activation wave results in the P wave, which has been discussed Figure 6A).

From the atria, the wave moves down the ventricular septum. It moves from left to right across the septum (Figure 6B on the following page). Since this direction points away from the positive electrodes of lead I, II, and III, they show an initial short downward deflection, called the Q wave (Figure 3).

Next, the electrical wave in the heart moves down the ventricular septum and through the ventricle walls, in an average left-and-downward direction (Figures 6C-1 and 6C-2). This is the main thrust of ventricular electrical electrical activity and constitutes the R wave.

The last parts of the ventricles to be activated are the upper portions (Figure 6D). The waves are weak and move to the upper right, and this causes a slight S wave.

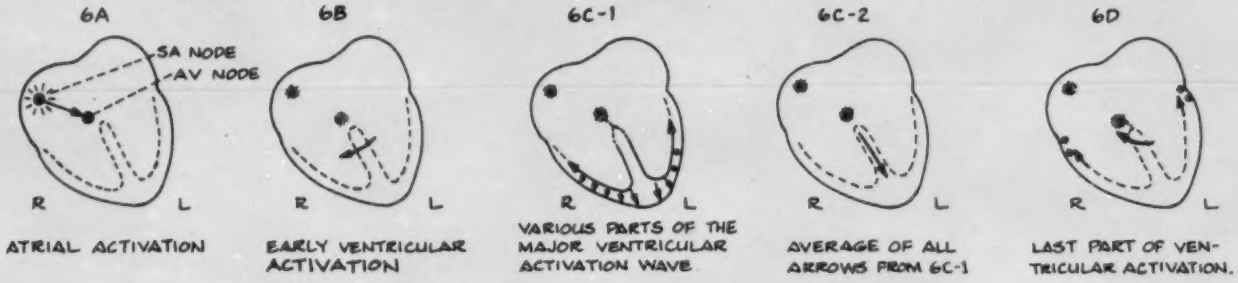


FIGURE 6: The average direction of the activation wave at different times during the cycle.

Taken together, the ECG picture of ventricular activation is called the QRS complex, which is very large. Its large size is consistent with the fact that the average activation wave for the ventricles is so large. After ventricular activation comes the repolarization wave, the T wave (Figure 3), when ventricular muscle cells establish anew the positive charge outside their membranes. (Atrial repolarization contributes very little to the pattern because it takes place during the QRS complex, the latter being so large in comparison that it masks the former.)

To summarize: the P wave is caused by atrial activation, the QRS complex by ventricular depolarization, and the T wave by ventricular repolarization.

How is the ECG Used to Detect Disease?

During the 1920's a great deal of work was done to find out how heart diseases change the ECG. We shall examine a few important diseases. There are three main types of heart problems that show up on the ECG: rhythm disturbances, conduction disturbances, and heart muscle alterations.

1. Rhythm Disturbances--In atrial fibrillation, the atria are said to look like a "bag of worms." The heart surface looks as if there were a mass of crawling worms just underneath it because it twitches rapidly all over. The cause is not known, but fibrillation may be due to electrical discharges from multiple points in the heart wall instead of from the SA node only (Figure 7).

Normal discharge from the SA node.

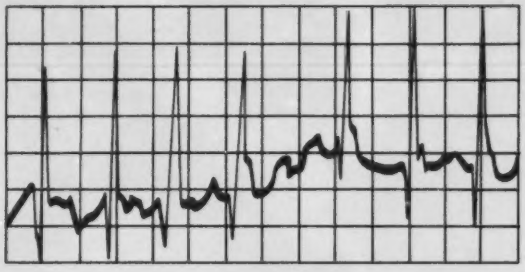
Abnormal discharges from many points, causing fibrillation.

FIGURE 7

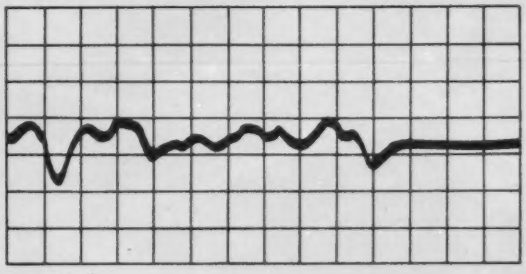
Fibrillation usually occurs in the atria rather than the ventricles. It occurs in a variety of heart conditions and can be treated with drugs. Electrical activity in the atria is so uneven that impulses from the atria reach the AV node at irregular intervals. This makes the AV node send impulses into the ventricles at irregular intervals, so that the ventricular rhythm becomes irregular. Figure 8A shows an ECG of atrial fibrillation

The ventricles can still get enough blood from the atria, even though the atria aren't beating normally, because the ventricles normally become 75 per cent filled with blood before the atria contract.

Sometimes the ventricles fibrillate (Figure 8B). This is the usual cause of death in myocardial infarction (death of heart muscle tissue due to loss of its blood supply) and is fatal in minutes because no blood is being pumped. Atrial fibrillation, on the other hand, is usually not fatal because the ventricles beat normally, although rapidly and at irregular intervals.



8A: The thick wavy line represents electrical discharges through the atria. The tall steep peaks (QRS complexes) represent ventricular activity. The peaks occur at uneven intervals.



8B: Ventricular fibrillation, ending in no heartbeat.

FIGURE 8

Ventricular fibrillation is treated with short electrical shocks applied to the chest. These shomehow override the ventricles' irregular electrical activity and give them a chance to resume a normal rhythm. Such electronic defibrillating devices are found in emergency rooms. They are used in conjunction with a technique called cardiac massage. Surgeons in the past opened the chest and massaged the heart to pump blood. Today, effective massage is provided simply by pressing down on the lower third of the sternum with the heel of the hand, about 60 times per minute. A person can be kept alive in this way for a half-hour or more until help arrives.

2. Conduction Disturbances--If the bundle of His is completely destroyed by an infarction, the activation wave from the atria can't get to the ventricles. The ventricles, however, have their own slow pacemaker activity, causing them to beat 20 to 35 times per minute. The atria, of course, still beat normally, so the ECG shows a normal P wave rate for the atria but less frequent QRS complexes for the ventricles. This is called complete heart block (Figure 9).

3. Heart Muscle Alterations-- When an artery supplying the ventricular muscle is suddenly occluded, infarction occurs to the muscle. The muscle supplied by the artery is now unable to re-establish its positivity. Hence the injured muscle area remains negative while the rest of the heart can and does re-establish its own positivity between ventricular beats. The injured negative area in the midst of the normal positive area between beats, causes a current to flow between ventricular beats when normally none does; this is seen in the ECG between QRS complexes as a deflection.

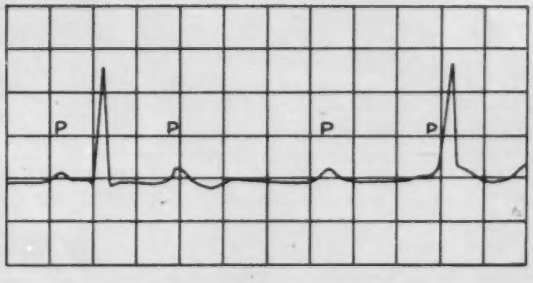


FIGURE 9: Complete destruction of the bundle of His (complete heart block).

Another ECG change due to muscle alteration is seen in people who have had high blood pressure for a long time. The left ventricle has to work harder to force blood into the arteries against the high pressure. Like any other muscle, e.g., the biceps in the arm, its mass increases to handle harder work. "Hypertrophy" is the technical term for mass increase. In left ventricular hypertrophy, more time is required for the activation wave to travel through the left than through the normal right ventricle because the left ventricle is larger. Therefore the left ventricle is still depolarizing after the right has finished, which shows up in the electrocardiogram. This, together with the fact that the larger-than-normal left ventricular mass results in a stronger than normal activation wave, causes a larger QRS complex (Figure 10).

Other things affect the way current is conducted in the heart, and change the ECG. These include especially the K^+ ion concentration of the body fluids. A change as small as ± 2 millimoles per liter can change the ECG, and greater changes can be fatal. Thyroid hormone deficiency also affects the ECG.

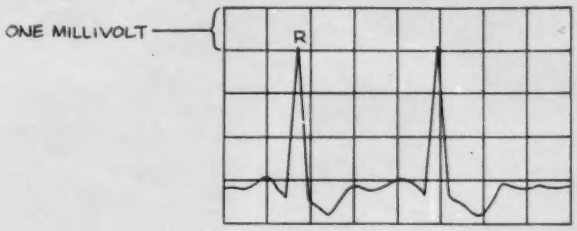


FIGURE 10: The Lead I pattern in left ventricular hypertrophy. Note the large R wave, of 3½ millivolts (normal is 1-2 millivolts). This represents a larger-than-normal ventricular activation wave.

INFORMATION ON LABORATORY ACTIVITY 8:

TEACHING NOTES:

1. The purpose of this activity is to study the cardiac cycle using the BIP to monitor the voltages associated with the heartbeat. Electrodes are placed on both legs and one arm, and the voltages monitored by the BIP are displayed with the BIP diode lights. The activity complements the discussion of the cardiac cycle and the electrocardiograph in ST-8.

2. Anticipated time: one to two periods. The activity may be augmented by adding any of the optional procedures discussed in Teaching Notes 7, 8 and 9.

3. The 18K-ohm resistor specified in the Procedure is supplied with two short green connecting leads as a standard BIP accessory item. The resistors are also available from electronics stores. They may be identified by their colored bands, which should be (in order) brown, gray, orange and silver (or gold).

4. Due to the design of the BIP, it is extremely unlikely that even a slight hazard could exist. If, however, any tingling is felt where the electrodes are attached (indicating a leakage of 0.1 to 1 mA of current) the subject should be disconnected immediately and the BIP should be checked (see Section 8 of BIP Manual).

5. Elastic bandages are available in 2-meter lengths at about $2.00 each. These may be cut into two 1-meter lengths. Other materials may be used to secure the electrodes such as gauze, masking tape or rags.

6. If electrode paste is unavailable, a 5% solution of NaCl on absorbent cloth serves as an acceptable (although messier) substitute. 200 ml of this solution should be enough for one class.

7. It is strongly recommended that the electrocardiogram be demonstrated first with an oscilloscope so that the students can view the shape of the cardiac wave as it is produced on an oscilloscope. This optional procedure (Activity 8-A) is detailed following the Answers to Discussion Questions. Using this procedure, it is possible to demonstrate the cardiac wave using the BIP diode display and the oscilloscope screen simultaneously. This approach should provide the students with sufficient background for the Student Procedure. If this demonstration is not undertaken, pictures and slides may be used as adequate preparatory material. An oscilloscope can usually be obtained from either a physics or electronics classroom. If extra oscilloscopes are available, the students may enjoy performing the activity (8-A) more than watching a demonstration.

8. A second option is included as Activity 8-B, in which the students place the electrodes on the chest and monitor the heartbeat using the BIP diode display. By moving the electrodes to different locations, the students can learn some of the effects of electrode position on the appearance of the cardiac waveform.

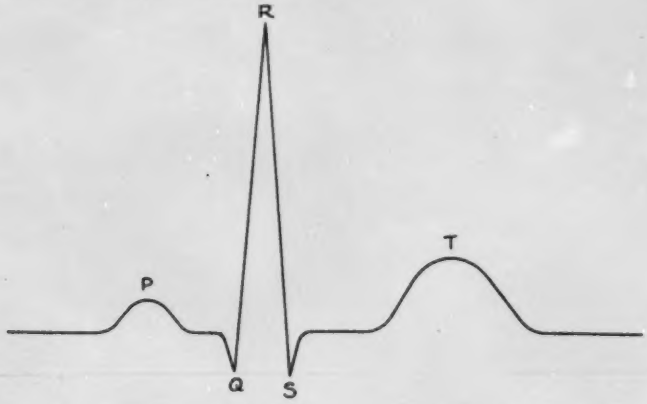
9. A third option is provided as Activity 8-C. In this procedure, the ECG is monitored using a loudspeaker. The tone generator of the BIP produces a continuous sound which changes with each heartbeat.

MATERIALS: (per 10 set-ups--not including options)

- 10 BIP's
- 20 electrodes with shielded cables
- 10 electrodes with unshielded cables
- 10 resistors, 18K-ohm, $\frac{1}{4}$-watt, attached to connecting wires
- 30 elastic bandages, 2 to 3 feet long
- 5 to 10 bottles acetone (or soap and water)
- cotton or cotton balls
- 2 tubes electrode paste (or 5% NaCl on absorbent cloth)

ANSWERS TO DISCUSSION QUESTIONS:

1.



2. P--contraction of the atria, QRS--contraction of the ventricles, T--prepares ventricles for next activation wave.

3. An electrocardiograph is a machine used for recording the changes in electric potential which are associated with the heartbeat. An electrocardiogram is the tracing of the cardiac waveform which is made by the electrocardiograph.

LABORATORY ACTIVITY 8-A: OSCILLOSCOPE DEMONSTRATION OF THE ECG PATTERN

INTRODUCTION:

In this demonstration, a volunteer is wired through the BIP to an oscilloscope so that the cardiac wave pattern may be displayed on the oscilloscope screen. The purpose is to demonstrate a normal cardiac wave pattern. The P, QRS and T waves should all be recognizable on the oscilloscope screen.

The demonstration should also introduce you to the BIP presentation of the cardiac wave pattern. The BIP provides the same information as the BIP oscilloscope, but in a different way. Thus, the BIP diode lights can also show the P, QRS, and T waves.

MATERIALS: (per set-up)

oscilloscope plus materials for Laboratory Activity 8

capacitor (if needed--see Step 9)

PROCEDURE:

1. Make the connections to the subject and the BIP as explained in Activity 8.

2. In addition, connect BIP pin C to the vertical input of the oscilloscope.

3. Connect pin X to the oscilloscope ground terminal.

4. Plug the BIP and the oscilloscope into grounded outlets, switch the oscilloscope on, and turn the intensity control high.

5. If there is a "60∿" switch on the scope control panel, make sure that you do not have it switched on.

6. a. Locate the knobs that control the location of the display on the screen. These may be called "V-pos" and "H-pos" or "vert. cen." and "hor. cent." Experiment with knobs until you locate the correct ones. The vertical-centering knob will move the display up and down. The horizontal-position knob will move the display right and left.

b. Adjust the two controls until the display is roughly in the center of the screen.

c. If all you get is a dot in the middle of the screen, the horizontal control must be adjusted as explained in Step 7. (A dot will sometimes burn the screen. Step 7 describes how to turn a dot into a horizontal line.)

7. a. Locate the knobs that control the horizontal dimensions of the display. Generally there are both coarse and fine controls for this. The labels for these controls vary from scope to scope. You might find them labeled "Horiz-Gain," "Horiz. Accenuator" or simply "Horizontal." You will know you have found the correct controls when you can vary the width of the display.

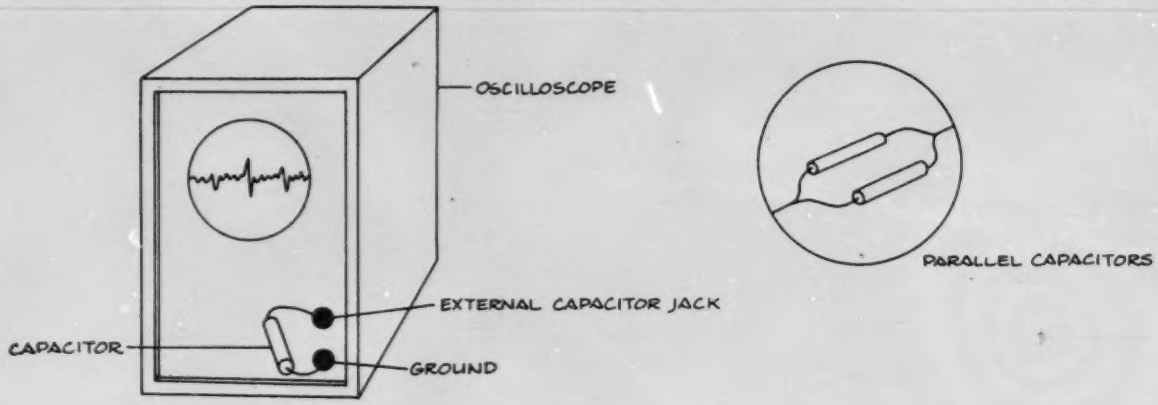
b. Adjust the width of the display until it just fills the screen.

8. a. Locate the controls for the vertical dimensions. The vertical controls will be analogous to the horizontal controls in the physical layout of the control panel, i.e., horizontal on the left implies vertical on the right. You will know you have located the right knob or knobs when you can vary the height of the display.

b. Adjust the display until it fills approximately half the vertical dimension of the screen.

9. The horizontal sweep of the oscilloscope should be quite slow (approximately one sweep per second). There are generally coarse and fine controls for the horizontal sweep frequencies. They may be labeled "sweep selector" and "sweep vernier" or "sweep attenuator." Or both controls may be combined under the cryptic label "frequency." You will know you have found the right controls when you can vary the rate of movement across the screen.

Many low-cost scopes will not provide a slow enough sweep. This can be corrected by attaching a capacitor between the scope's external capacitor jack and the ground (see figure). Try a 1.0 mfd (microfarad) capacitor with at least a 100-V rating. (If the capacitor has a + and/or - at one or both ends, be sure the negative end is connected to the ground.) If you need a slower sweep try a larger-value capacitor or additional capacitors in parallel with the first.



10. The ECG waveform should be visible on the screen at this point. If not, recheck all connections carefully, and consult the list of technical difficulties that follows. (If the height of the wave needs to be increased or decreased, adjust the BIP slotted gain control on the far right--#7 in the BIP diagram--to the desired level.) With the subject sitting quietly, the P, QRS and T waves should be visible. (The electrical activity of muscles is quite apparent if the subject contracts the muscles of his or her right arm.)

Possible Technical Difficulties:

1. If the subject is touching anything made of metal the waveform will move erratically on the oscilloscope screen. In our experience this has been the primary cause for poor ECG waveforms. It is imperative that the subject not touch any metal during the demonstration. Be sure to alert students to metal parts of desks and chairs.

2. Tenseness on the part of the subject will result in a waveform that has irregular peaks. The base line may also wander or be fuzzy. To eliminate the tension, make the subject as comfortable as possible. You might have the subject lie down on a pad or a cot. Unnecessary conversation should be avoided, as well.

3. Electrical interference is another commonly encountered difficulty. This is usually caused by radiation of alternating current from neighboring appliances or wiring (including fluorescent lights). One may sometimes alleviate this type of interference by disconnecting, or shutting off, unnecessary appliances near the BIP/oscilloscope set-up.

4. A wandering or jittery waveform (moving up and down erratically with quick, jerky movements) can be caused by movement of the subject and/or poor electrical contact. Advise the subject to sit or lie still and apply more electrode paste to the skin contacts. Paste which is contaminated with metal particles, or the presence of metal particles on the skin surface, can also cause a jittery waveform. Replacement of the contaminated paste and thorough cleansing of the skin should eliminate the problem.

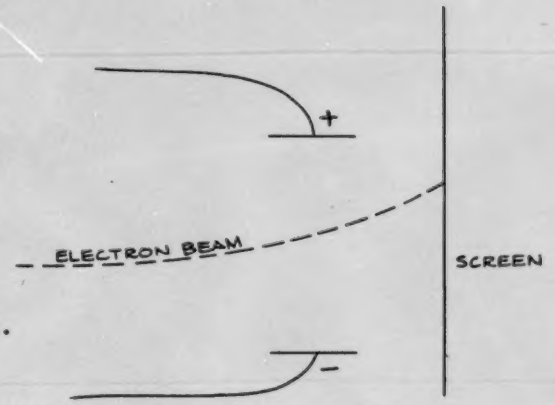
5. If the cardiac waveform appears upside down on the oscilloscope screen, reverse the electrode leads in the BIP input jacks.

INFORMATION ON THE OSCILLOSCOPE:

An oscilloscope converts electrical impulses into a visual display on its screen. It works as follows.

A beam of electrons is shot toward the screen. The screen has a fluorescent surface, and a spot of light appears where the beam strikes the screen. The vertical position of the spot is determined by two electrically charged plates, between which the electron beam passes.

If the upper plate has a positive voltage, as shown in the drawing, the negatively charged electron is attracted toward it and deflected upward. The voltage of the plates corresponds to the input voltage into the oscilloscope, from a source such as the BIP preamplifier or tone generator.



Two other plates, on either side of the screen, control the horizontal position of the electron beam. The voltage between these plates varies with time, so that the beam moves from left to right. When the beam reaches the right edge it returns to the left edge and begins its sweep again.

The time taken by the beam to make one sweep can be varied. If a signal of constant frequency is the vertical input, the horizontal sweep can be synchronized with the input so that the image on the screen remains stationary. If the signal and the sweep are not synchronized, the image will be seen to move across the screen.

LABORATORY ACTIVITY 8-B: ALTERING THE CARDIAC WAVEFORM

INTRODUCTION:

In Laboratory Activity 8, the cardiac wave pattern is studied with electrodes attached to the right arm and both legs. In this activity, one of the electrodes is placed on the chest. By moving the electrode to different locations, it is possible to show that the pattern of the cardiac wave changes on either side of the heart. The change in shape of the wave can be detected either with the BIP diode lights or the oscilloscope.

MATERIALS:

See Laboratory Activity 8 (and 8-A if oscilloscope display is desired).

PROCEDURE:

1. Make the connections indicated in Steps 1 to 8 of Laboratory Activity 8, except do not attach Electrode 2 to the left leg. If desired, connect the oscilloscope as indicated in Laboratory Activity 8-A.

2. Clean with acetone the areas indicated as X and Y in the figure.

3. Attach the free electrode at position Y, using electrode paste. To keep the electrode in place, have the subject hold the insulated portion of the electrode to the chest with a fingertip. The subject should move as little as possible. The metal portion of the electrode should not be touched with the fingers. Plug in the BIP.

4. Adjust the flashing diode display as indicated in Steps 10 through 14 of the Procedure in Laboratory Activity 8. Record your observations. Indicate any similarities or differences between the behavior of the cardiac wave pattern with

the electrode on the left leg and at position Y. If the oscilloscope is used, changes in the shape of the wave should be apparent.

5. Attach the electrode at position X, and repeat the observation of Step 4.

LABORATORY ACTIVITY 8-C: AN AUDIBLE ELECTROCARDIOGRAM

INTRODUCTION:

Normally, electrical signals from the heart are displayed visually by an electrocardiograph. The visual display may be seen on an oscilloscope screen, on a sheet of graph paper, or with the diode lights of the BIP. It is also possible to wire the BIP so that a sound is produced each time the heart beats. This audible signal may be produced at the same time that the BIP is producing a visual display.

MATERIALS:

See Laboratory Activity 8 (and 8-A if desired)

NPN transistor, small signal (such as 2N 3903)

small speaker, 8- or 16-ohm

resistor, $\frac{1}{4}$-watt, 150-ohm

2 BIP connecting wires, each with an alligator clip attached to one end

additional connecting wire, soldering iron, wire strippers, solder

PROCEDURE:

PART I: Preparing the transistor

1. Examine the transistor from the underside (with the wire leads). Notice that the body of the transistor has one side which is flattened (Figure 1). This flattened side serves to identify the transistor leads which are designated as shown in the figure.

2. Cut three wires into six-inch lengths.

3. Strip one end of each wire approximately one-half inch.

4. Strip the other end of each wire approximately one-fourth inch.

5. Solder the half-inch stripped end of one of the wires to the BASE (B) of the transistor.

6. Solder the half-inch stripped end of another wire to the EMITTER (E) of the transistor.

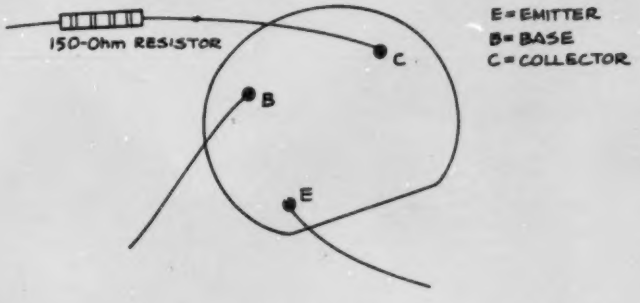


FIGURE 1: Transistor viewed from the underside.

7. Cut both leads of the 150-ohm resistor to half-inch lengths.

8. Solder the half-inch stripped end of the remaining wire to a lead of the resistor.

9. Solder the other lead of the resistor to the COLLECTOR (C) of the transistor. The transistor and the resistor should now be ready to attach to the BIP.

PART II: Programming the BIP

1. Make the connections indicated in Steps 1 through 8 of Laboratory Activity 8.

2. Attach the transistor - resistor assembly as shown in Figure 2 on the following page.

3. Use the two connecting wires with alligator clips to connect the speaker to the BIP as shown in Figure 2.

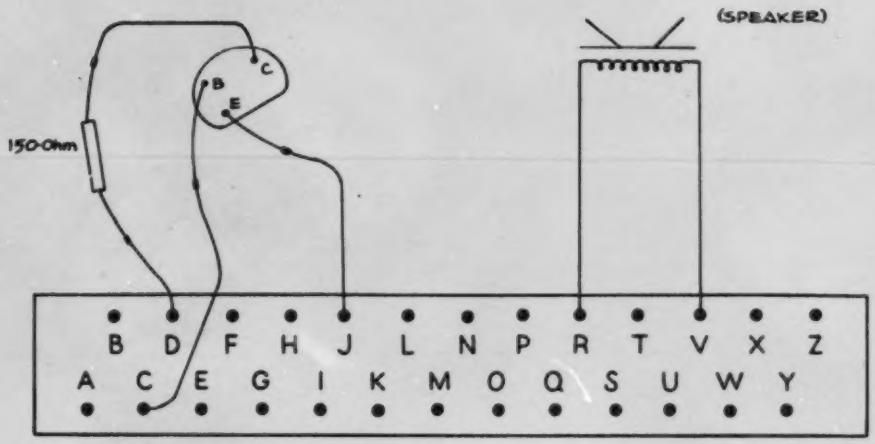


FIGURE 2: BIP program.

4. Set the frequency range switch (#9--just to the right of the mA dial) in the "up" position. Turn the amplifier gain control dial to zero (#2--at the left).

5. If desired, attach the oscilloscope to the BIP programming panel. Pin X is attached to the oscilloscope ground. The oscilloscope vertical input should be attached to the BASE (B) transistor lead which is connected to pin C. (A connecting wire with an alligator clip would be helpful.)

6. Plug the BIP (and the oscilloscope) into grounded outlets.

7. Increase the amplifier gain control setting until a tone is clearly heard. The tone should be steady except during each heartbeat. The QRS component of each cardiac wave should make a distinct "blip" in the tone.

8. The frequency of the tone may be varied as desired by adjusting the frequency control dial of the BIP (#6--at right). The mA dial may also be adjusted as explained in Laboratory Activity 8 to produce the flashing diode display.

ANTICIPATED RESULTS FOR LABORATORY ACTIVITY 8-B:

As the mA dial setting is decreased with the electrode at position Y, the diode display should be similar to the results obtained with the electrode attached to the left leg. An oscilloscope display will show a larger R wave, and possibly a larger T wave, for position Y than for the left leg. The similarity of the two results stems from the fact that both electrode positions are to the left and downward from the heart. The higher peak(s) at position Y is a result of the proximity of the electrode to the heart.

With the electrode at position X, the ECG waveform has a much different character (see figure). The difference can be more easily understood if the oscilloscope is used. With the electrode at position Y (or left leg), the main thrust of ventricular activation is toward the electrode and is reflected by a large positive deflection (the R wave). For position X, this same event has an average direction that is away from the electrode and produces a large negative deflection. (Note that this wave is labeled "S" in the figure. This seeming anomaly results from the following arbitrary definitions.

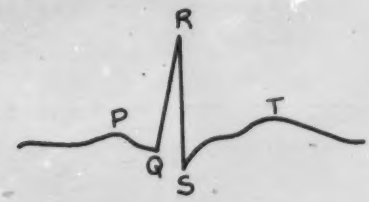
Q wave = first negative deflection after the P wave

R wave = first positive deflection after the P wave

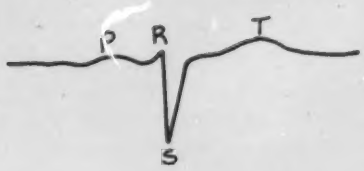
S wave = first negative deflection after the R wave

Using these definitions, it may be seen that the R wave for position Y is

equivalent to the S wave for position X. The Q wave for position Y is also equivalent to the R wave for position X.)



ECG pattern with electrode placed at position Y.



ECG pattern with electrode placed at position X

Using the BIP diode display, position X will in most cases give no flashes until the mA setting is lowered far enough to pick up the S wave. At that point, there should be one flash per heartbeat.

LESSON 9: (A) ATHEROSCLEROSIS AND HEART ATTACK

(B) REVIEW

RATIONALE:

Atherosclerosis, as an underlying cause in coronary heart disease (CHD), is examined in ST-9. The effects of this degenerative disease on the coronary arteries are emphasized, after which the symptoms of a heart attack are described. Two techniques useful in testing for coronary atherosclerosis are outlined.

As stated earlier, CHD is the leading cause of death in the U.S. in the age group 36 and over. This lesson reviews the importance of this disease while outlining a few diagnostic tests and precautions which could reduce risk. In Activity 9, the students determine the risk of CHD for four hypothetical individuals.

This lesson has relationships with earlier Science lessons (Unit II, Lessons 1, 2, 13, 16), Social Science lessons (Unit I, Lessons 12 to 15, 19 to 21) and Mathematics, Unit II, Lesson X. In addition, this lesson provides information which will be needed for Social Science, Unit III, Lessons 18 to 21.

OBJECTIVES:

The student will:

- define atherosclerosis and describe three distinct stages in its development.
- state the usual signs of myocardial infarction.
- distinguish between thrombosis and embolism.
- list at least three risk factors correlated with CHD infarction.
- evaluate risk of CHD when given case histories with data on risk factors.

SEQUENCE: ST-9; Activity 9; Review Set 9

SUGGESTIONS:

1. It should be emphasized that a lot is known about the way in which CHD develops. While there is not conclusive evidence to support the idea that environmental factors play a key role (e.g., diet, exercise, smoking, stress, etc.) these

factors are generally accepted as risks associated with the disease.

2. The inherent durability of the human body might be discussed in relation to this lesson. For example, individuals can lead relatively normal lives with a loss of 75% of the functional capacity of the heart. Such reserve functional capacity is characteristic of most body organs. Thus 85% of liver function may be lost without the appearance of symptoms, and many individuals function quite adequately with one kidney or one lung.

3. You may wish to point out that about 80% of the victims of myocardial infarction survive their first attack.

4. Because of the numerous relationships to earlier Science lessons (see Rationale), the current lesson provides an opportunity to review and reinforce earlier concepts such as the relation between nutrition and heart disease.

5. Because of the relationships with both Mathematics and Social Science, you may wish to discuss this lesson with your Biomedical colleagues in these disciplines. The ties to Social Science are especially important here because of the relation of this lesson to the Social Science poster contest (Unit III, Lessons 18 to 21).

INFORMATION ON ATHEROSCLEROSIS, ANGINA PECTORIS AND MYOCARDIAL INFARCTION:

I. Atherosclerosis:

This is the disease of arteries responsible for over half of all U.S. deaths. To understand the condition, let us first examine the microscopic appearance of arteries.

Normal arteries are composed of three distinct layers (Figure 1). The innermost is the intima, which is thin and consists of a layer of smooth endothelium and some connective tissue. Next is the media, or middle layer, of the artery. It consists of a thick, muscle-cell layer and some elastic tissue, the former enabling the artery to regulate blood flow by changing its diameter, the latter giving arteries a shock-absorbing capacity for the sudden force sent into them by each heartbeat. The third and outermost layer, the adventitia, consists of tough fibers that lend the arteries strength.

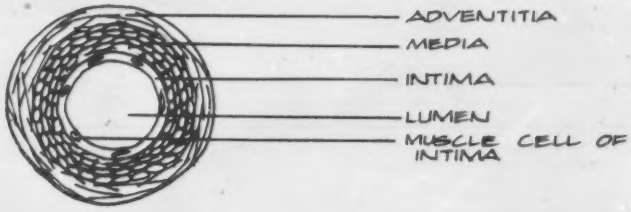


FIGURE 1: Normal artery cross section, X100.

A. Progress of Atherosclerosis:

This occurs in three stages.

1. First seen are fatty streaks which are flat, yellow areas. The color is caused by a lipid deposit (lipid is a general term that includes fats, fatty acids and cholesterol; refer to Nutrition Unit, Lesson 7). It occurs in the intima, especially in and around the intima's muscle cells. These muscle cells may manufacture the lipid deposits in response to substances, possibly cholesterol and fats, that travel in the blood. Fatty streaks have been found in almost all teenagers examined at autopsy and even in the aortas of some three-year-olds. Atherosclerosis begins early in life.

2. Fibrous Plaques develop from fatty streaks when a white cap of tough fibers grows over the lipid deposit, possibly a reaction by the intima to an irri-

tating effect of the lipids (Figure 2). The plaque protrudes into the vessel, narrowing it.

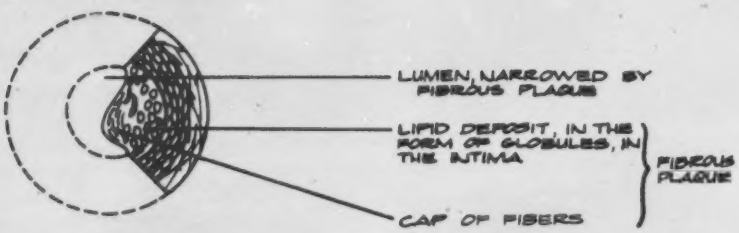


FIGURE 2: Artery with a fibrous plaque, X100, with only the segment containing a fibrous plaque drawn in.

3. This is the stage at which major problems occur. Complicated lesions ensue when the fibrous plaque is altered in one or more ways: by deposition of brittle calcium salts in it, by rupturing, or by being a base on which a blood clot (thrombus) forms. (The protruding plaque causes a slowing or a turbulence of blood flow, either condition tending to cause blood to clot.) A thrombus, as it enlarges, can further narrow a vessel. It can also break off and flow with the blood until it becomes lodged in a vessel too small for it to pass through, occluding the vessel. This is called an embolism.

Fibrous plaques can also weaken the media and adventitia by exerting pressure on them, making the vessel wall balloon out at that point; this ballooning is called an aneurysm. If it bursts, the blood that the artery carries escapes and exerts a dangerous pressure on tissues. Moreover, key nutrients are not delivered to some body site(s). Burst aneurysm and thrombosis are two of the main causes of stroke when they occur in the brain. (Hemorrhage due to hypertension is the third.)

B. Control of Atherosclerosis:

The incidence of CHD has been correlated with measurements of blood pressure, blood lipids including cholesterol, weight, skinfold thickness (thickness of the fat layer under the skin), lack of physical exercise, dietary lipids, cigarette use, dietary sucrose, number of cars per 100 people in a community, income, age, sex and personality type, among other things. (Women before menopause have fewer heart attacks than men their age, while the rate for women after menopause approaches that of men their age. This fact suggests a possible hormonal influence in atherosclerosis.)

There is no general agreement on which factors are most important, but there is considerable evidence that diet, in many people, plays a key role. As early as 1908, experimental animals fed high-fat diets were found to develop increased blood lipids and atherosclerosis more often than normally fed animals. A study conducted by WHO in the 1950's correlated the percentage of total calories supplied by saturated fatty acids with the incidence of cardiovascular disease (disease of the heart and blood vessels) in each of several countries. The people of east Finland and the U.S. obtain roughly 20 per cent of their calories from saturated fatty acids, and have four to six times the rates of cardiovascular disease as the people of Japan, Crete and parts of Yugoslavia and Greece. People in these countries obtain 10 per cent or less of their calories from saturated fatty acids.

It is important to emphasize that such studies imply, but do not conclusively prove a cause-and-effect relationship between dietary fat and atherosclerosis. Recently, however, a twelve-year study in Finland showed a definite decrease in cardiovascular deaths in people placed on low-saturated, high-polyunsaturated-fatty-acid diets.

It is important to note that atherosclerosis is probably due to a combination of many factors, e.g., someone who both smokes and has high blood pressure has a greater chance of experiencing a myocardial infarction than someone with only one of these factors.

Like tuberculosis, which became epidemic when social conditions changed in

the 19th century (overcrowded urban areas, poor ventilation), atherosclerosis may be an epidemic brought about by the changed social conditions of this century, such as the elimination of infectious-disease deaths, less exercise and different diets. A cause-and-effect relationship, however, has not been shown.

II. Angina Pectoris:

If an occlusion builds up gradually in a coronary artery, the heart muscle may not receive enough blood, and hence not enough oxygen, for maintaining increased pumping during exercise, e.g., walking up stairs. When coronary artery disease becomes very extensive, so that only the borderline amount of blood that can sustain the heart gets through, difficulties occur with minimal exertion or even at rest. The heart betrays its lack of oxygen at such times by transient pain in the chest, called angina pectoris. This pain typically has a sudden onset, occurs in the middle front of the chest, feels like constriction and, if one rests, stops in less than 10 minutes. It can be mistaken for indigestion because belching and stomach gurgling sometimes accompany it. Between attacks of angina pectoris, there is no other heart-related difficulty. Most people with angina will be found to have almost complete to complete obstruction of at least 2 of the 3 major coronary arteries. Survival under such conditions stems from the fact that the obstructions build up very gradually over a period of many years. As the flow of blood is reduced, there is an increase in the vascularization of the regions adjacent to the obstructed vessel. In effect, the obstruction is by-passed. The flow of blood through these alternate pathways, however, is never as great as through normal, unobstructed arteries.

III. Myocardial Infarction:

If a coronary artery suddenly becomes occluded by a thrombus, myocardial infarction (MI) usually occurs. ("Myocardial" refers to heart muscle; "infarction" means death of tissue due to lack of blood.)

An MI usually occurs unexpectedly and, unlike angina, is unrelated to whether or not the person is exercising. It almost always occurs when coronary artery disease is already present, but extensive disease is not a requirement. Thus younger people are also victims. The pain is more intense and prolonged than in angina; its onset is usually followed by profuse sweating, shortness of breath and nausea. The first few hours after onset are most important because cardiac arrest (ventricular fibrillation, a rapid uncoordinated twitching of the heart) occurs then, resulting in a seriously inefficient pumping of blood.

If death does not occur during the healing period, when the patient recovers the heart is left with a scar where the heart tissue died. The size of the scar is related to the extent of the original infarction. While the healed heart may still be a good pump, there is almost always some degree of lasting impairment.

Long-range treatment is based on controlling dietary fat and smoking, maintaining an optimum weight, treating high blood pressure (if present), and sometimes surgically replacing an occluded coronary artery with a healthy vessel from a part of the body that has enough other vessels to survive, e.g., the lower leg.

KEY--REVIEW SET 9:

1. a. Heart.

   b. Arteriole, artery, capillary, vein, venule.

2. a. Carries O_2 to cells, removes CO_2 and other wastes, carries nutrients to cells, regulates body temperature.

   b. Veins carry blood to the heart; arteries carry blood from the heart. Veins (except the pulmonary veins) are low in O_2, high in CO_2. Arteries (except the pulmonary arteries) are high in O_2, low in CO_2. Arterial walls are firmer and contain a thicker layer of muscle tissue. Blood pulsates through an artery, flows smoothly through a vein. Blood is at higher pressure in an artery than in a vein.

3. a. Valves only open in direction of the blood flow, towards the heart.

b. Capillaries connect the arteries with the veins and allow an exchange of materials between the bloodstream and the cells.

4. a. The pressure drops because energy is lost to friction.

b. The speed increases because of the reduction in the total cross-sectional area of the vessels.

5. By the expansion and contraction of arterioles.

6. Water flows from an area of greater water concentration (outside the cell, in the distilled water) to an area of lesser water concentration (within the cell). The influx of water causes the cell to burst.

7. Right atrium pumps blood to right ventricle; right ventricle pumps blood through pulmonary artery to the lungs; left atrium receives blood from lungs and pumps it to the left ventricle; left ventricle pumps blood through aorta to the body.

8. From the coronary arteries.

9. a. To listen to heart sounds.

b. Exercise, emotional excitement, food intake, drinking caffeine.

10. a. Systolic -- highest arterial pressure during each heartbeat.

Diastolic -- lowest arterial pressure during each heartbeat.

b. Hypertension is synonymous with high blood pressure. High blood pressure is medically important because it greatly increases the risk of heart attack and stroke.

11. It secretes a chemical that slows the heart rate.

12. Increases stroke volume; increases heart rate.

13. To record the electrical activity of the heart as an aid in diagnosing heart disease.

14. It regulates the pace of beating when the natural pacemakers are not functioning.

15. Diet, level of bodily activity, smoking, heredity, body weight, age, sex, etc.

16. Stage 1 - Deposits of fats and cholesterol in the walls of the artery. Stage 2 - plaque formation. Stage 3 - blood-clot formation.

INFORMATION ON ACTIVITY 9:

TEACHING NOTES:

1. This activity serves three purposes: (1) to review CHD risk factors discussed both in this unit and in Unit II, (2) to provide some information that will be useful for a poster contest activity in Biomedical Social Science, Unit III, Lessons 18-21 and (3) to emphasize preventive medicine.

2. Anticipated time: two periods.

3. The activity may be shortened by including fewer case histories or assigning one or more as homework.

4. It would be possible to extend the activity in several ways. For example, more case histories could be taken from the curriculum and scored for total risk. If this option is taken, you will need to give students supplementary information as we did in the activity. A second possible way to extend the activity would be to ask the students to make up additional case histories. Another possibility is to have the students assess their own risk. If they wish to do this, they may want

to know how to score themselves in regard to blood-cholesterol levels and saturated fatty acids. If they think that they have an average American diet, they can score themselves "0" for the risk associated with blood cholestorol and "1" for the risk associated with saturated fatty acids. If this option is elected, you may wish to point out that student scores should be low because of their age. It might be informative for students to calculate their risk of CHD after age 60 based on their present lifestyle.

5. It will be important to emphasize the distinction between a statistical relationship and a cause-and-effect relationship.

6. The calculations of total risk may vary a point or two because of the subjective nature of parts of the scoring procedure.

ANTICIPATED RESULTS:

	RISK SCORE			
FACTOR	Tom	Fred	Maria	Mrs. Jones
Reference (Unit and Section)	I, 41	II, 2	II, 6	II, 33
Age	4	6	2	3
Sex	5	5	1	2
Weight	2	3	0	3
Blood cholesterol level	3	4	1	4
% of total calories from saturated fatty acids	4	4	2	4
Systolic blood pressure	3	4	1	3
Tobacco smoking	10	6	10	0
Exercise	8	8	6	8
Family history	4	6	1	4
Total Risk	43	46	24	31

ANSWERS TO DISCUSSION QUESTIONS:

1. Highest risk: Fred; lowest risk: Maria.

2. Tom: Give up smoking, exercise a little more, watch your diet.

   Fred: Lose weight, quit smoking, get more exercise.

3. No. A higher percentage of people with a high total risk will develop CHD than people with a lower score, but not everybody with a score of 35 (or even 55) will necessarily have CHD.

4. No. (Same kind of argument as in 3.)

LESSON 10: (A) THE NATURE OF BLOOD

(B) DETERMINING HEMOGLOBIN CONCENTRATION

RATIONALE:

This lesson discusses the composition and properties of blood, the fluid component of the circulatory stystem. The subject of blood and its properties will be extended through Lesson 18, since the condition of the blood is one of the most important indices of health. The laboratory activities will demonstrate the clinical significance of the blood's composition. For example, LA-10 involves a measure of the hemoglobin concentration of blood. This analysis is used in the diagnosis of anemia.

OBJECTIVES:

The student will:

- list the cellular and non-cellular components of blood.
- distinguish between blood and plasma.
- list the following functions of the blood: transport, regulation of pH and temperature, resistance against microbial infection.
- list at least three symptoms of nutritional anemia.
- perform and describe the oxyhemoglobin procedure for measuring hemoglobin concentration in the blood.

SEQUENCE: ST-10; LA-10

SUGGESTIONS:

1. Students may want to know the answer to the question in Section 10-3. If all the erythrocytes of a person were laid out end to end, they would form a chain extending for about 1.2×10^5 km (131,000 miles).

$$24 \cdot 10^{12} \frac{\text{cells}}{\text{body}} \cdot \frac{1 \text{ cm}}{1.2 \times 10^3 \text{ cells}} \cdot \frac{\text{m}}{10^2 \text{ cm}} \cdot \frac{\text{km}}{10^3 \text{ m}} = 2 \times 10^5 \frac{\text{km}}{\text{body}}$$

2. Some of the complex terminology can be made easier for students by breaking terms into parts and defining the parts, e.g., erythrocyte (erythro = red, + cyte = cell) and leukocyte (leuko = white, + cyte). This approach can be extended to comparable medical terms such as erythema (a redness of the skin), erythromycin (a red antibiotic), a cytology (the study of cells) and leukemia (cancer involving excess white blood cells).

3. Somewhere in this sequence of nine lessons on blood you might wish to plan a field trip, (e.g., to a hematology laboratory) or invite a guest speaker to describe the activities of a blood bank.

4. Students may wish to organize a blood drive in their community or volunteer to assist at a mobile blood bank. Students eighteen and over (or seventeen and over with parental permission) are eligible to be blood donors (assuming they pass the blood bank's screening tests). If any of the students have donated blood, they might discuss the screening test. The test in which a drop of blood is allowed to fall into a cylinder containing a blue solution (copper sulfate) is to check blood density. If the blood settles too slowly, the person may be anemic and would not be an acceptable blood donor.

5. You may wish to ask whether any of your students are now or have been anemic. If so, they could describe their symptoms and treatment.

6. An interesting discussion question might be the following one "As a group, which would be more likely to be slightly anemic: (a) children, (b) females aged 15 to 50, (c) males aged 15 to 50, (d) females over 50 or (e) males over 50? Why?" (Answer: b. Most individuals in this group lose blood periodically through menstru-

ation. In addition, members of this group are the most likely to be pregnant and a mild case of anemia is sometimes associated with pregnancy.

7. Students may not understand what is meant by a biconcave disk. You can make a simple clay model of an erythrocyte by taking a disk of clay and pressing the center between your thumb and index finger.

8. Background information on blood and blood diseases follows. You may find various uses for this information. You may, for example, use the information to show some relations of Unit III to Unit I (gas exchange) or to Unit II (nutritional and pernicious anemia). The background information may also be useful in relation to the analysis of the upcoming laboratory activities.

9. Students may be confused about the difference between "serum" and "plasma," since the term serum has been used before. The distinction will become clearer after the discussion of blood clotting in Lesson 16. When clotted blood is centrifuged, the sediment is a blood clot and the straw-colored supernatant fluid is called "serum." Serum is very similar to plasma chemically. The two differ a little because, in the process of clotting, a few blood factors undergo changes. In particular, fibrinogen, a soluble blood protein of plasma, is converted during clotting into fibrin, an insoluble complex of proteins.

10. You may wish to remind students that formed elements do not refer to chemical elements.

11. You may wish to repeat the earlier activity on oximetry (Unit I, LA-36) or a similar activity in connection with the sequence of lessons on blood.

INFORMATION ON BLOOD AND ITS DISEASES:

Introduction

This is the first of nine lessons on blood and its functions. The first few lessons examine blood and other body fluids and the composition of blood. Microscopic examination of human blood introduces the students to the cellular composition of blood. Hemoglobin analysis and electrophoresis of blood serum introduces them to some of the protein components of blood serum. Then the immunological properties of blood are studied. The last few lessons consider the clotting and the buffering properties of blood.

Blood: A Brief Overview

Blood is a mixture of plasma and cells suspended in the plasma. The cells are: red blood cells (RBC), white blood cells and platelets (which are actually cell fragments). The following table shows the various transport functions of blood.

What Is Transported	Part of the Blood that Carrries It
Oxygen	Hemoglobin in RBC's
CO_2	RBC's, plasma proteins, plasma
Substances that protect against disease organisms and foreign substances	White blood cells and plasma proteins
Substances that function to seal off wounds to blood vessels	Platelets and plasma proteins
Hormones	Plasma
Fats and the fat-soluble vitamins	Mixed with globulins, which are plasma proteins
Minerals	Plasma and plasma proteins
Amino acids, sugars and water-soluble vitamins	Plasma

Oxygen and Hemoglobin

Oxygen is transported almost entirely by a special iron-containing protein called hemoglobin (Hb). You may wish to refer students to Unit I, Lesson 33, for a review of Hb and O_2 transport.

In the 1660's, before oxygen was discovered, Richard Lower observed that blood from veins turns bright red on exposure to air; now we know that when the oxygen in air combines with the Hb in RBC's, it forms bright red oxyhemoglobin. Iron oxide is not formed in this reaction; rather the oxygen remains as a molecule, O_2, and is only loosely associated with Hb's iron. RBC's are well adapted for gas exchange: they are relatively flat and thus more Hb is close to the cell surface than if the cell were spherical.

Note that most invertebrate animals don't have Hb. Lobsters, for instance, have a deep blue blood, the color being imparted by hemocyanin, a protein containing copper instead of iron. Most insects have no special oxygen-carrying substance at all; as a result their blood can carry only as much oxygen as will dissolve in the water contained in their blood and this is a relatively small amount. Human plasma can hold 0.3 ml oxygen per 100 ml plasma, while the Hb in RBC's enables blood to hold up to 20.0 ml oxygen per 100 ml blood.

One might wonder whether there is any advantage to having hemoglobin within cells rather than dissolved in the blood plasma. Consider what would happen if the hemoglobin were dissolved in plasma. The RBC's in 100 ml of blood contain 12 to 18 g of Hb, and 100 ml of plasma contains 6 to 8 g protein. Since Hb is a protein, if the Hb in our RBC's were dissolved in the plasma the total plasma protein would become 18 to 26 g/100 ml of plasma. This would make plasma so viscous that the heart couldn't easily pump it. Also, if Hb were not contained in the RBC's it would diffuse out of the bloodstream.

Some Statistics on Hb and Blood

1. Blood composes about 7.5% of the body mass in men and 7.0% in women. A 60-kg woman thus has about 4.2 liters of blood.

2. In men, about 46% of the blood consists of cells, mainly RBC's. This number, without the percentage sign, is called the hematocrit and sometimes the packed cell volume (PCV). The normal PCV range in men is 46 ± 5 and in women 41 ± 5. Thus blood is a bit more than half liquid (plasma), and the rest is cells.

3. Under normal conditions, the amount of Hb in blood varies with the PCV; in general, the more cells in blood, the more Hb in blood. Adult males typically have 14 to 18 grams Hb/100 ml blood, women 12 to 16.

4. Each RBC is about 34% Hb. This figure is smaller in many anemias, when RBC's have less Hb.

5. Men have about 5.4 million RBC's per cu mm of blood. Women have about 4.8 million. RBC's average 7.7 micrometers in diameter, somewhat larger than the average bacterial cell.

6. A gram of Hb can combine with up to 1.38 ml of oxygen; the Hb is then 100% saturated. When blood leaves the lungs the Hb saturation is usually about 97%; the Hb in venous blood is 75 to 80% saturated. In strenuous exercise, however, venous blood can be only 10 to 20% saturated because blood gives up so much of its oxygen to muscle.

Hb and Anemia

The blood hemoglobin determination is probably the most commonly performed quantitative determination in clinical medicine. That is because the Hb level is affected by so many conditions. A Hb concentration below a certain level indicates anemia and above a certain level indicates polycythemia. It is important to remember that these are not diagnoses but are "signs" of an underlying medical problem.

Anemia is more common than polycythemia and is probably present at some time

in the life of most women and about a third of the male population. The most common types of anemia are listed in the table below.

	Cause	Blood Smear
Decreased RBC Production	Fe deficiency	small, pale RBC
	Folate deficiency Vitamin B_{12} deficiency	large RBC
	Bone marrow damage	granulocytes often very low.
	chronic illness/infection	
RBC loss	Hemorrhage --acute such as in trauma --chronic--usually from GI tract due to ulcer or tumor	
Increased Destruction	Certain chemical agents and poisons. Intracellular abnormalities.	

In most anemias, the hemoglobin is of the so-called "A" or adult type. In sickle-cell anemia, which cannot be fit logically into the above table, the Hb molecule is altered at only one amino acid linkage. The altered hemoglobin, called the "S" type, results in cells that change shape in the presence of low O_2 tension. These episodes of sickling are often associated with tissue damage and pain. This disorder will be considered in more depth in a later unit on genetics.

In connection with the Hb determination in LA-10, it might be well to stress the importance of precise measurement in clinical medicine. Consider a laboratory that has poor quality control and an imprecision of ± 1.0 g for Hb. For women the usual normal range is 12.0 to 16.0 g/100 ml. If 14.2 were reported, we could be confident that the patient has sufficient Hb, because 13.2 to 15.2 is still well within the normal range. But what if the reported result were 12.2? This patient could be well within the normal range at 13.2 or definitely anemic at 11.2 and needing further study. Conversely, a result of 11.5 might result in unnecessary and possibly costly study when, in fact, the true Hb level was 12.5.

It seems shocking now that for years many physicians relied on a visual estimation of the color produced by a drop of blood on white blotter paper. The error of this method was about ± 4 g Hb/100 ml or at least 25 per cent.

Polycythemia is more common than generally realized. It frequently occurs in heavy cigarette smokers or people with chronic lung disease, especially emphysema. Physiological polycythemia occurs to some extent in most people who spend several days or longer at elevations above 8,000 feet. Generally, polycythemia is a compensatory state. If the hemoglobin content of the blood becomes too high (over about 19 g/100 ml) the blood becomes too viscous, so the net effect may be of no physiological advantage. In fact, polycythemia may lead to a type of hypertension.

Iron-Deficiency Anemia

In adults iron-deficiency anemia is rarely due solely to a dietary lack of iron, because meat and vegetables have abundant iron, and foods low in it, such as cereals, are often enriched with iron. Rather, it is usually caused by a loss of blood over a long period of time, as in heavy menstrual bleeding or ulcers, such blood loss represents the loss of iron which would normally be recycled in the body. Anemia due to blood loss is best treated by stopping or reducing the bleeding as well as

giving iron tablets. It is important to realize that it is the loss of the iron in blood that causes the anemia. If such people replace the iron lost, anemia does not develop, because the marrow can usually make enough Hb and RBC's to replace the ones lost, as long as it has enough iron.

Why does the loss of blood deplete the body of iron? Blood has a lot of iron: 3.4 mg per gram of Hb. Since women have about 14 g of Hb per 100 ml of blood, their blood must have 48 mg iron per 100 ml. Menstrual blood loss is usually 20 to 60 ml/month, which means an iron loss of 10 to 29 mg/month. The requirement for menstruating women is that they absorb about 2 mg of iron per day, or about 60 mg/month, from the diet, which is sufficient to replace the 10 to 29 mg lost in blood and compensate for other losses as well. If the diet is low in iron and bleeding heavy, the body can eventually, sometimes over a period of years, lose enough iron for anemia to occur. It can take years because there are iron stores in the liver and spleen.

For this reason, some birth control pills have iron added as a sure way to supplement the diet. The RDA for iron is 18 mg (for both sexes), of which only 10% is absorbed, or about 2 mg. Cheap wines often contain a lot of iron, so poor alcoholics are not only rarely anemic, but they sometimes develop siderosis (the Greek word for iron is "sideros"), an accumulation of excess iron throughout the body.

Vitamin B_{12} and Folic Acid-Deficiency Anemias

Both vitamin B_{12} and folic acid are needed by all cells to make DNA. Almost all cells developing into blood cells in the marrow are duplicating. But before they can divide they must first make a certain amount of DNA, since every new cell must inherit a complete set of DNA. In B_{12} and folate deficiencies, erythroblasts (and other cells) take a very long time to make enough DNA to divide, and during this extra time each cell has a chance to grow much larger than normal. When marrow cells that are developing into RBC's become abnormally large they are called megaloblasts (meaning "big sprouts"). Thus, B_{12} and folate-deficiency anemias are called megaloblastic anemias. The marrow cells which grow into white blood cells also become larger due to vitamin B_{12} and folic acid deficiencies. Indeed, in this kind of anemia abnormally large cells are found in all parts of the body where there is normally a lot of production of new cells. The RBC's associated with vitamin B_{12} and folic acid-deficiency anemia have normal amounts of Hb but are large and often oval-shaped instead of round. The megaloblasts are destroyed by special phagocytic cells in the marrow before they can become RBC's. Therefore fewer than normal RBC's are produced, resulting in anemia.

Folate-deficiency anemia occurs mostly in pregnant women because the fetus uses a lot of this vitamin. Discovered by Lucy Wills in the 1930's, the vitamin's name comes from folium (leaf) because it was first isolated from leafy vegetables, which are rich in it. B_{12}-deficiency anemia is also called pernicious anemia (PA) because it was fatal until it was found in the 1920's that eating half a pound of liver a day cured it.

B_{12}-deficiency anemia, unlike folate-deficiency anemia, is hardly ever due to a dietary lack of this vitamin but instead usually due to a lack of a special protein made in the stomach, called intrinsic factor. This protein must bind to the B_{12} in food for the intestine to absorb B_{12}. Patients with B_{12} deficiency don't make this intrinsic factor; they usually don't make stomach acid either. It is thought that antibodies somehow turn against the person's own stomach cells, destroying them so they can't make intrinsic factor or stomach acid. B_{12}-deficiency anemia is a hereditary disease, and is now easily treated by B_{12} injections. It may also occur in infants who are breast fed by strict vegetarian mothers, for B_{12} is lacking in vegetables, its main sources being meats and dairy products.

The Effects of Anemia

A diminished quantity of Hb in the blood results in the blood's being unable to carry enough oxygen. The lack of O_2 affects the vasomotor center in the medulla of the brain. This center in turn directs the heart to push blood through the body faster to make up for the oxygen lack in blood; the heart beats faster, the blood pumped per beat increases, and the person may feel his heart pounding, a phenomenon called palpitation. Other effects due to decreased oxygen levels include frequent fatigue, dizziness, faintness, headache, and tendency to feel cold. There can also

be pallor, best seen in the nails or mucous membranes. Some of these symptoms were included in the case history in Section 10.

If anemia is very severe and prolonged, the heart can actually fail from oxygen deprivation.

A Word on Some Other Anemias

Radiation and certain chemicals can destroy the marrow. These agents include benzene, some of the drugs used to kill cancer cells, and radioactivity. Some substances directly destroy RBC's in large doses: benzene, toluene, lead and some snake venoms. Anemia is also common in cancer, rheumatoid arthritis and other long-lasting illnesses and may be caused by the inability to recycle iron properly from dead RBC's back into the marrow.

INFORMATION ON LABORATORY ACTIVITY 10:

TEACHING NOTES:

1. This activity is the first of a series on the physiology of blood and the use of blood in the diagnosis of disease. In this activity the students use the oxyhemoglobin method to measure hemoglobin concentration in a blood sample. This method is accurate and still in use in clinical laboratories.

2. Anticipated time: one period.

3. The calibration line for hemoglobin determination was prepared using blood samples of known hemoglobin concentration. As with calibration lines used earlier in the course, it is based on a "typical" BIP. BIP-to-BIP variation will, of course, lead to some error in the results obtained. In our experience, this error is slight. If you are concerned about this you might obtain a sample of blood of known hemoglobin concentration from your local hospital and test it on each BIP. If the value you obtain using the calibration line given deviates more than 0.5 g/100 ml from the known value, you can adjust the position of the line as appropriate for the BIP in question.

4. It is recommended that about a third of the class perform the finger puncture in this activity and provide enough blood for the entire class. Another third of the students can later provide blood for LA-11, and the rest of the students can provide blood for LA-13. In this way, each student has to make only one puncture, rather than three. If this recommendation is followed, some advance coordination of activities is necessary so that the other lab groups are ready when the punctures are made.

5. You might caution the students to dispose of lancets as soon as they have performed the punctures, to prevent their re-use. This precaution is taken to avoid the spread of infectious diseases such as veneral disease and hepatitis.

6. Be sure you have checked on any district, county or state regulations concerning students making finger punctures. Make sure you have any required permission forms or other procedures taken care of in advance.

7. $EDTA\text{-}Na_4$ solution is used in this procedure rather than water because it:

(a) binds any metallic ions which are present in the water and thus prevents these ions from causing the breakdown of hemoglobin;

(b) provides the alkaline environment necessary for all the hemoglobin to be converted to oxyhemoglobin.

8. In the Introduction to LA-10, the students are given the structural formula of $EDTA\text{-}Na_4$. You may wish to point out that the name used for this chemical (tetrasodium salt of ethylenediaminetetraacetic acid) does not correspond with the nomenclature rules they learned in Unit I. The proper name is ethane-1, 2 diaminetetraacetic acid, tetrasodium salt. Unfortunately, there are many such reagents still being marketed under names derived from now-obsolete nomenclature systems.

9. In the Laboratory Manual and in the Information on Laboratory Activities, we specify "alcohol cotton wipe packets" whenever blood is obtained. These packets are marketed as "cotton swabs."

10. For good quantitative results in this activity, it is necessary to hemolyze the red blood cells. This is accomplished by placing the blood in a hypotonic EDTA-Na_4 solution. Water rushes into the red blood cells causing them to swell and then burst (hemolyze).

We have never completely explained to students how cells react to hypotonic and hypertonic solutions. A good point for such an explanation would have been in Lesson 3, and perhaps you made the necessary distinctions at that time. If not, we recommend that you take the time to explain the concepts now in relation to the activity.

The salient points follow. When a cell is placed in a hypotonic solution (i.e., a solution that is dilute with respect to the inside of the cell), water tends to rush into the cell. The water moves from a region of high water concentration (outside the cell) to one of lower water concentration (inside the cell). The process may also be described in terms of osmotic pressure. In this instance, the osmotic pressure inside the cell is greater than outside and water is forced in. (Keep in mind that osmotic pressure is defined as the pressure necessary to prevent the movement of solvent into a solution.) Conversely, when a cell is placed in a hypertonic solution (i.e., a solution that is concentrated with respect to the inside of the cell), water tends to move out of the cell into the solution. The water moves from a region of high water concentration (inside the cell) to one of lower concentration (outside the cell). The osmotic pressure is greater outside the cell than inside the cell.

The response of cells to hypotonic and hypertonic solutions is essentially the same for solutions of large molecules such as proteins, small molecules such as sugars, or ions such as Na^+. The latter situation may puzzle students. If a cell is placed in distilled water, the students may wonder why ions do not leave the cell while the water enters the cell. The truth is that both occur but the cell membrane is more permeable to water than to ions or molecules. The cell membrane is selective in regard to diffusion, and the selectivity is not simply based on size. Due to the selectivity of the cell membrane, water molecules move in and out of a cell much more readily than other substances. Thus, it is convenient to explain the effect of hypotonic and hypertonic solutions solely in terms of water diffusion.

MATERIALS: (for 30 set-ups)

- 1.5 g EDTA-Na_4
- 10 to 15 sterile blood lancets
- 10 to 15 alcohol cotton wipe packets
- 30 micropipets, 20-µl (disposable)
- 30 pipets, 10-ml
- 60 cuvets, 16 x 125 mm, Pyrex
- 10 BIP colorimeters
- 15 glass-marking pencils
- cotton or tissues

PREPARATION OF REAGENTS:

0.3% solution of tetrasodium salt of ethylenediaminetetraacetic acid (also under the names of (ethylenedinitrilo)-tetraacetic acid tetrasodium salt and versene). Abbreviated formula: EDTA-Na_4

Add 1.5 ± 0.1 g EDTA-Na_4 to sufficient tap water to make 500 ml of solution. EDTA-Na_4 dissolves readily in water and can be stored at room temperature for at least two months. (The chemical is not harmful in small quantities. Rinse mouth with water if any is accidentally drawn into the mouth during pipetting.)

ANTICIPATED RESULTS:

When this test was performed in our laboratory the following values were obtained.

Male: 15.5 g Hb/100 ml blood (average of 3 males)

Female: 15.0 g Hb/100 ml blood (average of 2 females)

ANSWERS TO DISCUSSION QUESTIONS:

1. Depends on student results. Most results should be in the normal range.

2. It may be possible to demonstrate that the male average is higher. The normal ranges are 14 to 18 g Hb/100 ml blood for males and 12 to 16 g Hb/100 ml blood for females.

LESSON 11: (A) BLOOD CELLS

(B) BLOOD SMEARS

RATIONALE:

The appearance and relative proportions of blood cells are important diagnostic clues to a variety of clinical conditions. In LA-11, the students learn how to prepare blood smears. In ST-11, the significance of blood smears is considered. In addition, ST-11 discusses the structure-function relation in red blood cells (RBC's). The basic concept of cell differentiation is introduced to explain the different stages in the development of RBC's. Cell differentiation will be treated in some depth in a later unit on reproduction and genetics.

OBJECTIVES:

The student will:

- define and give at least one example of cell differentiation.
- compare and contrast RBC's, leukocytes and platelets by making blood smears using the Wright-stain technique.
- explain how a RBC is adapted to its function.
- list at least two clinical conditions that may be detected by blood smears.

SEQUENCE: ST-11; LA-11

SUGGESTIONS:

1. The lesson follows very closely on the text of the previous lesson. It may be a good idea to review the case history in ST-10 before discussing the continuation of that case history in ST-11.

2. You may wish to expand on our treatment of RBC differentiation. Background information on this topic is provided below.

3. Leukocytes are introduced here because of the clinical implications of the laboratory activity. The leukocytes will be reconsidered in relation to later lessons on immunity, phagocytosis and blood clotting. At that time, the functions of neutrophils and lymphocytes will be examined.

The background information on leukocytes that follows should not be discussed with the students until Activity 14 has been completed. In addition, a supplementary subsection is included which you may wish to reproduce for your students. This subsection is intended to follow Section 11-4 and is concerned with infectious mononucleosis, a common condition in young people.

4. You may wish to supplement this lesson with charts or 35-mm slides showing the types of blood cells found in normal or abnormal blood smears. Such materials may be obtained from biological supply houses.

SUPPLEMENT TO SECTION 11:

11-5 Infectious Mononucleosis and Lymphocytic Leukemia

How can infectious mononucleosis be detected in a blood smear?

Infectious mononucleosis is a common disease of people in the 15- to 30-year age range. It's probable that you either have had a bout with mononucleosis (or "mono" as it is often called) or have friends who have had mono. Mono may begin like a bad cold--sore throat, fatigue, headaches--but often it soon gets much more unpleasant, and it may last for several weeks or months.

In the diagnosis of mononucleosis, blood smears are routinely required. The Wright stain shows a high count of leukocytes (see Figure 4)--typically up from the normal 3,000 to 10,000 per mm^3 to about 10,000 to 20,000 per mm^3. More significantly, the increase is mostly of lymphocytes, which become 60 to 90 per cent of the leukocytes.

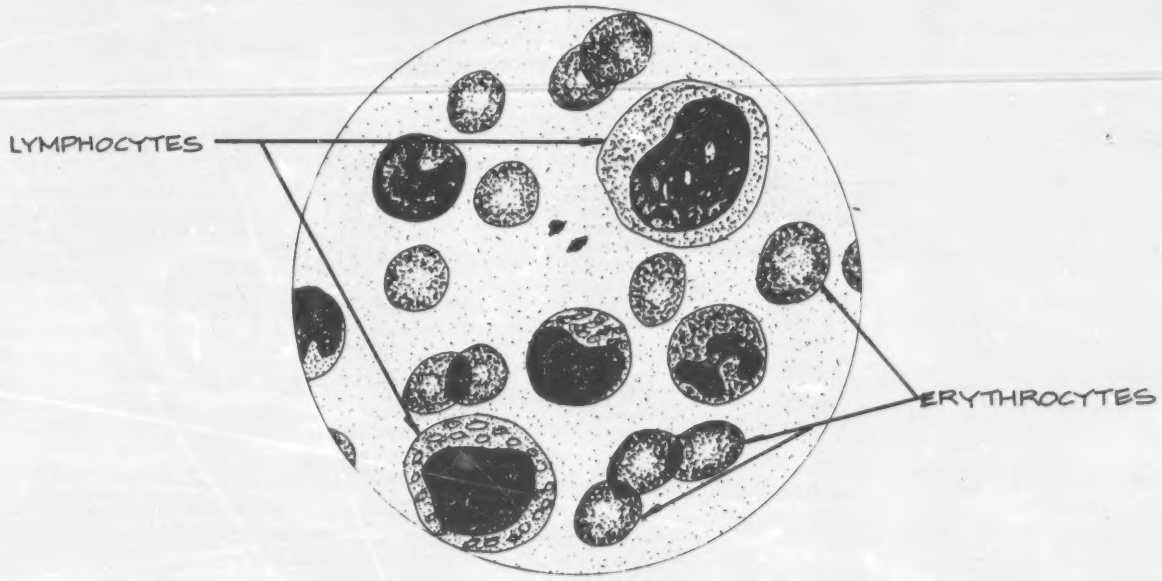


FIGURE 4: Blood smear typical of infectious mononucleosis.

The pathologist who analyzes the Wright stains must take pains not to confuse infectious mononucleosis with other blood diseases, especially lymphocytic leukemia ("leuko" means "white" and "emia" means "blood"). Lymphocytic leukemia is a fatal cancer that also results in excessive production of lymphoctyes. In this form of cancer, the Wright stains show very high leukocyte counts (often over 100,000 per mm^3) and the lymphocytes have unusual shapes. Sometimes bone-marrow smears are made to confirm the diagnosis.

INFORMATION ON LEUKOCYTES AND DIFFERENTIATION OF ERYTHROCYTES:

Phagocytosis: An Introduction

In 1882 Elie Metchnikoff saw ameba-like cells in a starfish larva surround a rose thorn with which he had pricked it. He called the cells phagocytes, because they are cells which "eat" foreign substances and bacteria. Some cells in our body are phagocytic. The ones in the blood are leukocytes or white blood cells (WBC's). Of the five major classes of WBC--neutrophils, monocytes, eosinophils, lymphocytes and basophils--only the first three are phagocytic. All WBC's show ameboid motion. There are normally about 3,000 to 10,000 WBC's per mm^3 of blood.

Function of the Leukocytes

Neutrophils phagocytize bacteria, mostly outside of the bloodstream because

bacteria rarely enter the blood. There are plasma proteins called "complement" and "immunoglobulins"--they interact with bacterial products to attract neutrophils out of the bloodstream and into the infected zone. Neutrophils leave the blood by squeezing through tiny gaps in capillary walls. This may seem unlikely, since the smaller RBC's cannot pass through the capillary walls. However, the larger neutrophils are able to change their shape (like an ameba). This property enables them to literally "flow" through small openings in the capillary walls. When many neutrophils collect in an infected area they compose pus, which also contains bacteria and debris from dead cells of the area.

It is not enough for a neutrophil to ingest a bacterium. It must then kill it, for otherwise the bacteria can continue reproducing inside a neutrophil and burst it open. The neutrophils' killing agent is hydrogen peroxide (H_2O_2). In the presence of an enzyme in the neutrophil called peroxidase, the H_2O_2 oxides bacteria to death. There is a rare disease in which neutrophils lack peroxidase and so can't kill bacteria. It is called granulomatous disease of childhood. Its victims die of infection.

Monocytes are second only to neutrophils in phagocytic activity. They often clean up debris at the end of infections, and are a second line of defense after neutrophils, following them into an area. Monocytes also pass through openings in the capillary walls, via the same mechanism used by neutrophils.

Eosinophils increase in the blood during parasitic infections, especially with certain worms, and commonly also in allergies such as hay fever. In allergies, they may phagocytize foreign proteins contained in dust and pollens.

Lymphocytes are an important part of the immune system. We will examine them carefully in a later lesson.

Basophils contain heparin, which is an anti-clotting agent, and histamine, which attracts neutrophils into an injured or infected area.

Structure of the Leukocytes

Leukocytes are grouped into two classes: granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes), according to whether there are granules in the cytoplasm. Note, however, that lymphocytes and monocytes do actually contain a few granules, but far fewer than the granulocytes.

In all of the following discussion it should be understood that descriptions of color refer to the color after the application of Wright's stain, a mixture of eosin, which is red, and methylene blue in alcohol.

Lymphocytes. Most of the lymphocytes in blood are small cells, about the size of RBC's or a bit larger. These make up 20 to 25% of the WBC's. The nucleus takes up most of the cell and is dark red to purple; the cytoplasm is blue. A small portion of lymphocytes are larger--2 to 3 times the size of RBC's. The lymphocytes in blood are actually a mixture from two different sources. The thymus gland in the chest produces short-lived lymphocytes while the lymph nodes and other lymphoid tissue in the tonsils, spleen, marrow and intestine make ones that live for months and years. The two types cannot be distinguished with Wright's stain. We will examine the functions of these two lines in a later lesson on immunology.

The lymphocytes in blood constitute only 1/25 of all the lymphocytes in the body. Most are in the thymus and lymph nodes. Lymphocytes can develop back into stem cells (to be discussed shortly) in order to give rise to the other kinds of blood cells: it is also thought that they can turn into monocytes when needed. Cells capable of transformation into other kinds of cells are said to be multipotential. Stem cells are multipotential because they give rise to RBC's, WBC's and platelets.

Monocytes. These constitute 2 to 10% of the total WBC's, are 3 to 4 times the diameter of RBC's, and have much more cytoplasm relative to the nucleus than lymphocytes. The nucleus is lighter than in lymphocytes and often sausage or kidney shaped. The cytoplasm is white. Monocytes have been seen, in rats, to develop from lymphocytes during infections.

The three types of granulocytes, discussed on the following page, have abundant

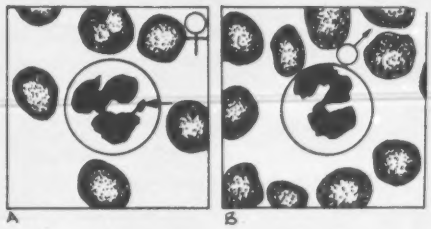
granules in their cytoplasm; and their nuclei are constricted into several lobes.

Neutrophils make up 50 to 75% of the WBC's. They are 1.5 to 2 times the diameter of RBC's, and their nuclei are long and twisted into 3 to 5 lobes. From a normal count of 2,000 to 5,000 per mm^3 of blood, their number can go up to 30,000 and higher in infections, perhaps a response by the bone marrow to bacterial products. This is important in diagnosing bacterial infections such as strep throat. (In viral infections, such as mononucleosis, the lymphocyte count, rather than the neutrophil count, often goes up.) There is a large reserve of neutrophils in the marrow, which is released during emergencies. The lifespan of neutrophils is 6 to 11 days. Wright-stained neutrophils have a red-purple nucleus and a whitish cytoplasm.

About 3% of neutrophils in women have a small extra piece of chromosome attached to the nucleus. This is a sex chromosome, and in clinical cases of hermaphroditism is used to determine the patient's genetic sex.

Basophils are the least prevalent WBC's, constituting less than 1% of the total. Their cytoplasm is peppered with large blue granules that contain the anti-clotting factor heparin. The nucleus is red.

Eosinophils constitute 2 to 5% of the WBC's and have a dark-staining nucleus and large red granules spread throughout the cytoplasm.



The "drumstick," an extra piece of nuclear material, actually a sex chromosome, in women's neutrophils (arrow).

Differentiation of Erythrocytes

All blood cells begin as stem cells, which are large cells that show a blue cytoplasm and a large, light-purple nucleus, on staining. As the stem cell matures into a RBC, these changes occur: the nucleus becomes progressively smaller and darker, the cell as a whole becomes smaller and the cytoplasm becomes lighter.

When stem cells turn into cells with a specific function, such as RBC's or lymphocytes, they make different proteins. As stem cells differentiate to form red blood cells, the key new protein manufactured is Hb. However, only a small portion of the DNA in the nucleus is involved in synthesis of Hb. Much of the rest of the DNA is bound to a basic protein known as histone. (The bound DNA is inactive.) Basic proteins react with the methylene blue in Wright's stain to produce the blue color seen in blood smears. Cells that are actively producing Hb are rich in histone and thus stain dark blue. The nucleus appears darkest blue at the stage shown as "C" in the Student Text, p. 33.

In contrast to the nucleus, which becomes darker during differentiation, the cytoplasm of developing RBC's becomes lighter, because there is a decrease of basic proteins in the cytoplasm. These basic proteins are used in hemoglobin synthesis. After a significant amount of Hb synthesis is completed, the nucleus is expelled and the basic proteins in the cytoplasm disintegrate. The cytoplasm in lymphocytes, however, remains blue because the basic proteins in the cytoplasm of these cells do not disintegrate. These proteins play a role in the production of antibodies throughout the life of the lymphocytes.

Returning to the differentiation of RBC's, at a late stage called the normoblast, the cell expels its nucleus. This is the only cell in the body that does so. The result is more room for the Hb it has made. The average RBC stays in the circulation for 120 days, which means that every day 1/120, or 0.8% of the RBC mass is destroyed and replaced. A protein hormone made in the kidney, called erythropoietin, increases the RBC production in the marrow. It is secreted in response to the loss of blood and also to the low oxygen at high altitudes. Thus it helps to maintain homeostasis.

INFORMATION ON LABORATORY ACTIVITY 11:

TEACHING NOTES:

1. This activity is the second in a series of activities on the properties of blood and its use in the diagnosis of disease. In this activity the students apply Wright's stain to a blood smear. With a microscope, erythrocytes, leukocytes and platelets may be distinguished. Students are also asked to compare the smears they make with commercial slides showing abnormal blood samples representing different diseases.

2. Anticipated time: one period.

3. It is not necessary for each student to make a puncture for each of the activities requiring blood. It is recommended that one-third of the class perform the finger puncture for Part I of this activity and another third make a puncture to provide blood for LA-13. In this case, each puncture should provide one drop of blood each for three different smears and thus service three different students. Clarify for the class which students will be making punctures at this time, and emphasize the need for the remainder of the class to be ready when the punctures are made. If a student knows he or she has some blood abnormality, his or her blood smear may prove interesting.

4. Note that in Part III of the procedure a second set of punctures is made in order to obtain blood samples for use in LA-13. In this case, only 10 punctures are necessary (i.e., one per BIP). However, up to 20 (two per BIP) blood samples can be analyzed in the electrophoresis activity, if desired. Students who have not made an earlier puncture should provide the blood for LA-13. The stored blood samples will keep for 4 days under refrigeration.

5. Any common dishwashing detergent can be used for washing slides. If you do not want your students to wash slides, have your lab assistant clean them prior to class. The clean slides could then be stored in jars containing 95% ethyl alchohol and dispensed directly from the jars as needed. Dirty slides can be placed in another jar of 95% ethyl alcohol prior to being cleaned. If the students clean their own slides, the amount of alcohol needed could be reduced by providing a common beaker of alcohol for all students to use.

6. You may wish to reinforce the caution in the procedure about not getting Wright's stain on clothes, books, tabletops, etc.

7. It may be possible to obtain abnormal blood cell slides free of charge from a hospital or private laboratory. Call the medical technologist of the facility well in advance of your needs. Identify your specific needs, i.e., slides showing sickle-cell anemia, iron-deficiency anemia, pathological leukocytes, etc., and describe how the slides will be used.

8. You may wish to make up a slide of your own blood prior to the activity so that the students can see what their smears should look like when completed. Alternatively you might obtain a pre-prepared microscope slide or a 35-mm slide of a normal blood smear from a biological supply house.

9. The quantities of materials called for in the Materials list assume that a third of the class provides blood for Part I and a third provides blood for Part III. The quantities are also based on the assumption that each student will make a blood smear.

10. You may have noted that the students are not asked to look for platelets. There are two reasons for this. First, platelets will not be properly considered until ST-16. Secondly, these structures are difficult to locate and identify, especially for a beginner.

MATERIALS:

0.2 g Wright's dye

6.63 g potassium phosphate, monobasic (KH_2PO_4)

15 microscopes

dropper bottles for Wright's stain and phosphate buffer ("pH 6 solution")

2.56 g anhydrous sodium phosphate, dibasic (Na_2HPO_4)

120 ml methyl alcohol

200 ml 95% ethyl alcohol

20 to 30 alcohol cotton wipe packets

20 to 30 sterile blood lancets

60 microscope slides

30 beakers, 50-ml

30 Petri dish covers or bottoms (or other glass containers to hold beaker)

10 to 20 micro-hematocrit tubes heparinized

lens paper

lint-free cloths (optional)

silicone rubber cement

masking tape

prepared microscope slides of abnormal blood cells

prepared microscope slide of normal blood smear (optional)

35-mm slide of normal blood smear (optional)

35-mm projector (optional)

PREPARATION OF REAGENTS:

Wright's Stain: Prepared Wright's stain can be purchased from most biological supply houses. If you would like to prepare your own, use the following recipe.

0.2 ± 0.05 g Wright's dye

120 ± 1 ml methyl alcohol

With a clean mortar and pestle, grind the dye with alcohol, adding the alcohol very slowly until all of the dye is in solution. Place in a dark bottle, allow to santd at least 24 hours and filter. Store in dark dropper bottles. Storage life: 6 months.

Phosphate Buffer: (pH = 6)

6.6 ± 0.1 g potassium phosphate, monobasic (KH_2PO_4)

2.6 ± 0.1 g anhydrous sodium phosphate, dibasic (Na_2HPO_4)

Add water to 1000 ± 10 ml. Label "pH 6 solution." Storage life: indefinite (if refrigerated).

ANTICIPATED RESULTS:

1. Comparison of different blood-cell types.

APPEARANCE	NAME	DIFFERENTIAL FEATURES
See ST-10,11	Erythrocyte	Nucleus absent; reddish-orange in color; round or disk-shaped; very abundant.
See ST-10,11	Leukocyte	Large round or multilobed nucleus; granules present or absent in cytoplasm; nuclear granules stain blue, purple or red; variable shape; few in number; larger than erythrocytes.

2. The normal ranges for erythrocyte and leukocyte counts are:

erythrocytes...4,000,000 to 5,000,000/mm^3

leukocytes...3,000 to 10,000/mm^3

Thus the ratio of erythrocytes to leukocytes is between 1700:1 and 400:1.

ANSWERS TO DISCUSSION QUESTIONS:

1. Debris on the slide or lens may obscure blood cells or be mistaken for blood cells by students.

2. Too much blood would lead to a slide with too many blood cells. The cells would overlie one another and would be difficult to study.

3. Answers will vary.

4. Answers will vary.

LESSON 12: (A) BODY FLUIDS AND EDEMA

(B) ELECTROPHORESIS

RATIONALE:

ST-12 considers the compartmentalization of body fluids in cells, blood vessels and interstitial space. It extends from certain principles of diffusion covered in ST-3 and elsewhere and leads to a consideration of edema, a clinical condition involving an excess of interstitial fluid. Edema may be a symptom of a number of disorders ranging from protein malnutrition to heart disease. In a later lesson, edema and body fluids will be related to the lymphatic system.

In LA-12 the technique of electrophoresis is introduced. The clinical implications of electrophoresis will be explored in Lesson 13.

OBJECTIVES:

The student will:

- list several important biological properties of water.
- explain what is meant by "compartmentalization of body fluids" and list the three compartments that house body fluids.
- define edema and list at least four causes of this condition.
- describe how edema can occur as a result of capillary damage.
- explain the diagnostic importance of edema.
- explain what a diuretic is and how a diuretic affects edema.
- separate two amino acids by means of electrophorsis.

SEQUENCE: LA-12; ST-12

SUGGESTIONS:

1. The electrophoresis activity is lengthy, so it is suggested that you begin LA-12 before ST-12. During the waiting period in the activity, you might discuss ST-12 or assign reading to the students.

2. In Section 12-1, it is pointed out that body water content "depends on age, sex and other factors." Students may be interested in the following table which summarizes the relation of age and sex to body water content in the United States. Since there is an inverse relationship between the amount of fat tissue and water content, a possible interpretation of the table is that (1) females tend to contain more fatty tissue than males and (2) both males and females tend to become more obese as they age.

BODY WATER CONTENT (%)

Age	Males	Females
5- 9	61.5	61.5
10-16	59.0	56.0
17-39	60.0	51.0
40-59	55.0	48.0
over 59	54.0	46.0

3. The treatment of the biological importance of water is very brief here, since the subject was discussed in Unit II, Lesson 10. You may find this a good time to review the properties of water based on the earlier discussion.

4. You may wish to discuss the advantage of having three compartments rather than one. There are organisms such as amebae and bacteria that are unicellular and thus they have only one compartment. In addition, there are animals such as the coelenterates that have two compartments--a layer of cells and an internal sac. However, in the larger organisms, there is a need for an efficient, rapid means of transporting substances to and from cells that are far from the region of absorption. This is provided by a three-compartment system.

The following points about compartmentalization may be made.

(a). An efficient means of internal transport is necessary when gas exchange cannot take place over the entire body surface but is confined to special respiratory organs (lungs or gills).

(b). A large fluid-filled body cavity can assist internal distribution of materials in a very small, simple animal but becomes inadequate if the animal is large, has a high metabolic rate, or the body cavity is crowded with organs.

5. Edema is common enough that close relatives of students may have had this condition. Such students may have interesting clinical details to fill in about symptoms and treatment.

6. The old name for edema is "dropsy." It comes from the Greek word for water.

7. The relationship of severe heart disease to edema is quite complex. The problem generally begins in the left ventricle which, for one reason or another, is inadequate in discharging blood. One frequent cause is coronary disease causing cardiac muscle weakness. Other causes are prolonged hypertension and valve disease which results in a mechanical inefficiency of the heart. In any event, severe heart disease has several effects: (1) the pressure rises in the left atrium, (2) the pulmonary veins enlarge and (3) increased pressure occurs in the lung vessels.

The increase in pressure in the lung vessels is transmitted back to the right ventricle via the pulmonary artery. It is actually this whole chain of events that generally results in an increased venous blood pressure and edema.

There is still a little more to this story. The increased pressure in the inferior vena cava somehow stimulates the adrenal cortex to secrete more aldosterone, a steroid hormone. Aldosterone causes the kidneys to reabsorb sodium ions which, in turn, leads to reabsorption of water by the kidneys. This means that less water is excreted in the urine. This increases the pressure in the blood vessels which aggravates the edema. So the original problem in the left ventricle leads to edema by two different mechanisms.

It should be clear from this discussion why an explanation was not provided in the Student Text. The explanation here is intended primarily for your own information.

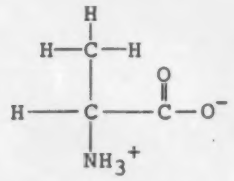
8. There is reference in Section 12-2 to the relationship between edema and the content of water and sodium ions in urine. The function of the kidneys in regulation of the concentration of water and other materials will be considered in Lesson 20.

BACKGROUND ON ELECTROPHORESIS:

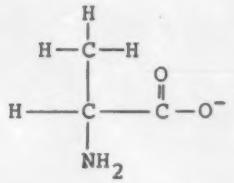
Electrophoresis is a technique developed during the past 25 years mainly for the study of proteins and enzyme systems. A popular support medium for research has been polyacrylamide gel which gives very good resolution of migrating proteins. In recent years, cellulose acetate has become increasingly popular in hospital labs, since it is non-toxic, easy to work with, and gives reasonably precise results in a short time. The procedure has traditionally been expensive, although in recent years some low-cost systems have been developed.

The degree of success that the students have will depend largely upon their technique, particularly in regard to their care in spotting the samples on the strip. When too much sample is applied, the results will be indistinct and difficult to interpret. If capillary tubes or micropipets are not available, try a toothpick as an applicator.

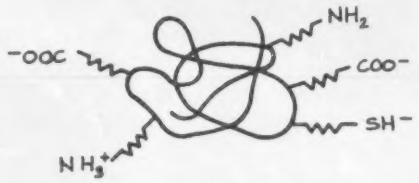
With a buffer at pH 9.0, most amino acids and blood proteins have a net negative charge as in the figure below. Thus they move toward the anode. Blood proteins are typically soluble and can be thought of as loosely coiled chains with the amino acid side chains sticking out from the surface.



Alanine (at pH 7)

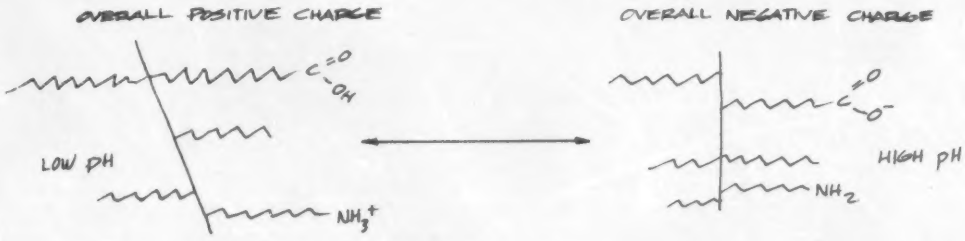


Alanine (at pH 9)



Protein (at pH 9)

At low pH, globular proteins are typically positively charged, since protons can combine with (COO^-) and amino groups of the amino acids (see figure). At high pH, the (COO^-) groups predominate, which confers an overall negative charge on the protein. (Albumin moves more rapidly than the globulins mainly because it has a large negative charge but also because it is less massive than the globulin molecules. The mobility of a molecule is also affected by the shape of the molecule, the ionic strength of the buffer, and the voltage.)



INFORMATION ON LABORATORY ACTIVITY 12:

TEACHING NOTES:

1. The purpose of this activity is to introduce the process of electrophoresis, a technique of diagnostic importance for a variety of medical conditions. It is also hoped that by practicing the techniques involved in electrophoresis, the quality of results in the electrophoresis of plasma proteins (LA-13) will be improved.

It should be emphasized that the purpose of this activity and the next is not to develop mastery of electrophoretic technique--it would take at least weeks to become proficient at the technique and would require the use of much more sophisticated equipment to achieve optimal results. The purpose is simply to introduce the

technique and indicate its usefulness. Along these lines, students should not be disappointed if the patterns they obtain are unlike those shown in ST-13.

2. Anticipated time: two periods, including a 45-minute wait.

3. This activity must be completed in one day, since there is no point in the procedure at which an interruption may be made.

4. In LA-12 the term "ionic solution" is used as a substitute for "buffer solution." This was done to avoid introducing a new term which might require a lengthy explanation. Buffers will be taken up in Lesson 17.

5. The electrophoretic (cellulose acetate) strips should be placed in the buffer solution about 15 minutes before they are needed. The purpose of the soaking is to make sure that the strips are saturated with buffer solution.

6. The amino acid solutions should be labeled "A" and "B." In the answers to the discussion questions, we assume that glutamic acid is "A" and glycine is "B."

7. The two solutions that make up the chlorinating mixture for developing the amino acids must not be combined until just before they are to be used. This is because chlorine is produced only for a short time. The two solutions should be kept in separate containers until the students require them.

8. The o-tolidine used in this activity is labeled as carcinogenic. However, this refers to quantities many times greater than those used in this activity. Normal precautions about handling toxic chemicals should suffice.

9. One developer bath and two baths of 5% acetic acid solution will be sufficient for the entire class. These can be set up in dissecting trays.

10. The procedure used for staining the amino acids is based on the fact that when amino acids are exposed to chlorine gas they form chloramine derivatives. When the chloramine derivatives are dipped in a solution containing potassium iodide and o-tolidine, the chlorine on the chloramines causes the o-tolidine to be oxidized. It is the oxidized form of o-tolidine that is responsible for the final bluish-black color of the spots. The potassium iodide serves merely to increase the sensitivity of the reaction.

11. The containers used for chlorinating the electrophoretic strips should be containers that can be covered in some way such as with a Petri dish cover. Covers are necessary to avoid too much release of chlorine gas in the classroom. Quart-sized containers are the most convenient, although pint jars may be used.

12. It would be useful to label the two acetic acid baths "1" and "2" to ensure that all students use first one and then the other.

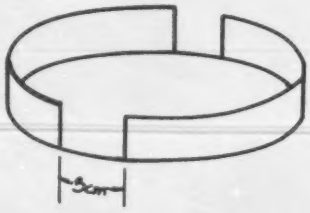
MATERIALS: (for 10 set-ups)

- 32.6 g sodium borate ($Na_2B_4O_7 \cdot 10H_2O$)
- 96 ml HCl, concentrated
- 30 ml acetic acid, glacial
- 0.5 g glycine
- 0.5 g glutamic acid
- 2.5 g potassium iodide (KI)
- 1.8 g o-tolidine
- 4.7 g potassium permanganate ($KMnO_4$)
- 10 to 20 cellulose acetate strips, Gelman 51003, SP E4710-3
- 20 microscope slides
- 20 beakers, 150-ml
- 10 lengths of wire, ~30 cm
- 20 medicine droppers
- 20 capillary tubes, 40-µl
- 10 BIP's
- 20 electrophoresis electrodes
- 20 glass tubes (18 cm length, 9 mm I.D.)
- 10 1000-ohm resistors
- wire, 24-gauge, insulated
- cellophane or masking tape
- silver-tin solder ("soft silver")
- paper tissues
- 4 dissecting pans (or similar containers for soaking acetate strips and for developer and acetic acid baths)

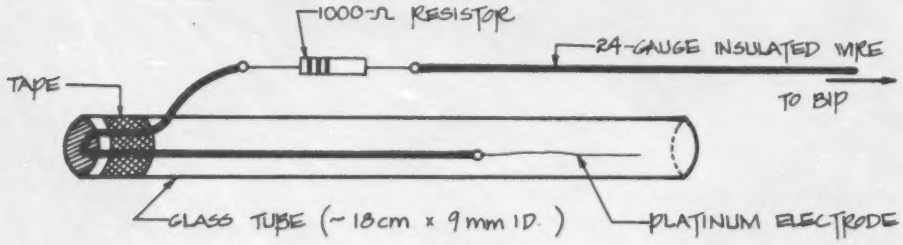
10 graduated cylinders, 100-ml
10 "cut-out" Petri dishes (plastic)
10 Petri dish covers
10 mm rulers
10 ring stands
20 ring-stand clamps
10 quart or pint containers with lids (see Teaching Note 11)

PREPARATION OF MATERIALS:

1. To make the "cut-out" Petri dishes, heat a razor blade held wiht forceps or heat a scalpel balde in the flame of a gas burner. Use the hot blade to cut the plastic Petri dish bottom as shown in the figure below. (The students could perform this step. If you have studetns do this, the Petri dishes should be prepared before the remainder of the procedure is begun.)



10. The electrodes may be made as shown in the diagram.



The platinum electrodes are made by Gelman Co. (#51136) and are distributed by Scientific Products (SP E4702-5). The electrode should be soldered to the wire with a silver-tin ("soft silver") solder using an appropriate rosin. Ordinary electrician's solder (60-40 rosin core) will not make a good joint. Note: Only one of the electrodes in each set needs to have a resistor connected. The resistor is primarily a safety precaution should a short circuit occur. Normally very little current flows. If there is a short circuit, the resistor will keep current flow minimal. The color code for a 1000-ohm resistor is brown, black, red and gold (or silver).

PREPARATION OF REAGENTS:

Borate buffer, pH 9.1 (Ionic Solution):

Solution A: 32.6 ± 0.1 g sodium borate ($Na_2B_4O_7.10H_2O$) + sufficient water to make 3500 ± 50 ml solution.

Solution B: To 400 ± 10 ml water add 8.6 ± 0.1 ml conc. HCl. Dilute with water to a total volume of 1000 ± 25 ml. (Note: This is a 0.1 M HCl solution.)

Buffer: Combine all of Solution A with 496 ± 25 ml of Solution B and dilute to a total volume of 6200 ± 100 ml, with water. Check the pH--it should be near 9.1. (Note: The buffer will suffice for this activity and LA-13. It should last at least one week if refrigerated. If a repeat of the activity is planned, new buffer should be used.) If the pH is off by more than 0.1 pH unit, adjust by adding dilute acid or dilute base.

2% acetic acid: 30 ± 2 ml glacial acetic acid + sufficient water to make 1500 ± 25 ml solution. Use 500 ± 25 ml for each acetic acid bath and 300 ± 25 ml for developer bath preparations.

Developer bath:

Solution A: 2.5 ± 0.1 g KI + sufficient water to make 300 ± 25 ml solution.

Solution B: Saturate 300 ± 25 ml of 2% acetic acid with O-tolidine (about 1.8 g). Mix well and filter.

Developer: Mix solutions A and B in a 1:1 ratio. Refrigerate. Shelf life of unmixed solutions: about 1 week. For storage, keep solution A and B in dark bottles. They may be mixed within an hour of the activity.

Chlorinating mixture: (Note: Solutions A and B should not be mixed in advance.)

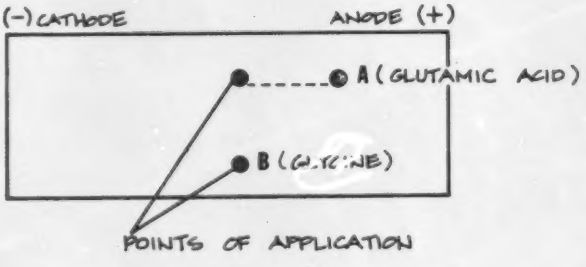
Solution A: 4.7 ± 0.1 g $KMnO_4$ + sufficient water to make 300 ± 25 ml solution.

Solution B: Add 87 ± 1 ml conc. HCl to 200 ± 10 ml water. Dilute with water to a final volume of 300 25 ml.

Amino acid solutions: For each solution dissolve 0.5 ± 0.01 g of the amino acid in 100 ± 5 ml water. Shelf life: Several weeks if refrigerated.

ANTICIPATED RESULTS:

We obtained the following results.



ANSWERS TO DISCUSSION QUESTIONS:

1. Amino acid A migrated toward the anode, while amino acid B did not migrate.

2. The amino acids did not migrate the same distances from their points of application. Amino acid A (glutamic acid) possesses a net negative charge, while amino acid B has a net charge of 0.

3. The functional group responsible for the migration noted is the COO^- group. There are two COO^- groups and one NH_3^+ group in amino acid A (glutamic acid), giving it a net charge of -1. (Note: The students are not familiar with the notation $-NH_3^+$. You may wish to explain that this group is the result of the following reaction: $-NH_2 + H^+ \longrightarrow -NH_3^+$.

4. Amino acid A is glutamic acid; it possessed a net charge of -1 due to the presence of two COO^- groups and one HN_3^+ group. Amino acid B is glycine. Glycine did not migrate because its net charge was zero.

5. This labeling was necessary so that the direction of migration could be interpreted in terms of net positive and negative charge.

LESSON 13: CLINICAL APPLICATIONS OF ELECTROPHORESIS

RATIONALE:

In LA-12, the technique of electrophoresis was introduced, along with a brief mention of its clinical value. In this lesson, some applications of electrophoresis in the diagnosis of disease are considered.

OBJECTIVES:

The student will:

- perform electrophoresis of plasma proteins.
- list at least three plasma protein fractions detectable by electrophoresis.
- define densitometry and explain why it is important in the clinical application of electrophoresis.
- point out the protein abnormality in a disease such as cirrhosis when shown electrophoretic patterns representing the disease and the normal state.

SEQUENCE: LA-13; ST-13

SUGGESTIONS:

1. Note that LA-13 should be completed prior to assigning ST-13, because ST-13 discusses the results of the activity.

2. Cirrhosis is referred to in ST-13 as an example of a disease for which electrophoresis may aid in diagnosis. The purpose is not to discuss cirrhosis in detail in this lesson. However, if students are curious about the disease you may find the background information on cirrhosis useful, or you could suggest that they refer to an encyclopedia.

3. In cellulose acetate electrophoresis of plasma, five distinct stained bands of proteins are generally seen. However, you may wish to explain to your class that there are many more than five plasma proteins, for each band is composed of two or more distinct proteins. Actually there are other proteins that migrate to positions between the stained bands but are at concentrations too dilute to detect. At any given time, an individual may have as many as several hundred different proteins in blood plasma.

4. Problem Set 13 should be straightforward for students who have mastered ST-13. For some students, it may be helpful for you to demonstrate how to analyze one electrophoretic pattern and have them do the other three by themselves or in groups.

5. It will be necessary to reproduce several pages of material in classroom quantity for Activity 14.

INFORMATION ON CIRRHOSIS OF THE LIVER:

Cirrhosis of the liver is a term often used to refer to any of several diseases of this organ characterized by tissue scarring. In cirrhosis, the shape of the liver is distorted and the liver becomes fibrous. (Portal circulation is typically impeded to some degree.) Cirrhotic patients are likely to complain of weakness and fatigue, a slight loss of weight and may show such signs of edema as swollen ankles. The edema results from a decrease in plasma albumin and consequent diffusion of water from the circulatory system into the interstitial space.

The death rate for liver cirrhosis has been rising and has become a major medical concern. It is now the eleventh leading cause of death in the U.S. and ranks fifth as a cause of death in American males between the ages of 25 and 64. Although it was once a disease found far more commonly in males than females, cirrhosis is becoming equally prevalent in females. At present, about 2 or 3 per cent of American adults have this disease.

A strong statistical relationship exists between liver cirrhosis and an excessive intake of alcoholic beverages. Poor nutrition is often associated with alcoholism and both are believed to lead to cirrhosis.

The treatment of alcoholic cirrhosis consists mainly of lowering or eliminating consumption of alcoholic beverages. In addition, a nutritious diet (including a restriction of salt intake) and bed rest is part of the therapy. The edema may be treated with diuretics.

There are other forms of cirrhosis, but they are less common than alcoholic cirrhosis. These other forms include cardiac cirrhosis (associated with severe heart disease) and biliary cirrhosis (caused by inflammation of the bile ducts).

KEY--PROBLEM SET 13:

A. hypogammaglobulinemia; B. chronic infection; C. nephrotic syndrome; D. normal

INFORMATION ON LABORATORY ACTIVITY 13:

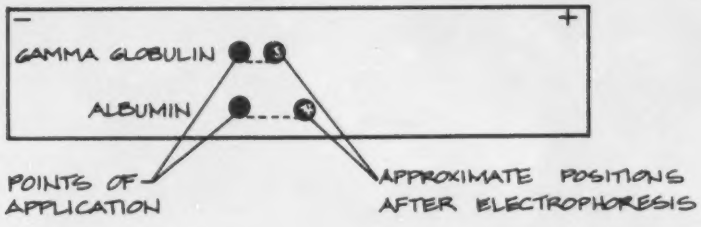
TEACHING NOTES:

1. The purposes of this activity are (a) to reinforce the techniques introduced in LA-12 and (b) to demonstrate the separation of plasma proteins into albumin and gama globulin fractions upon electrophoresis.

2. Anticipated time: two periods, including a one-hour wait.

3. As with LA-12, the cellulose acetate strips should be placed in the buffer solution about 15 minutes before they are needed.

4. If you wish to decrease the cost of the activity, you may omit the use of albumin and gamma globulin standards. If this is done, the following diagram of a sample electrophoretic strip should be made available to the students. Otherwise they won't be able to answer the discussion questions.



5. Since the students have had no previous experience with the transfer technique described in Procedure Step 7 of Part I, it may be useful for them to first practice the technique with water, using capillary tubes and paper towels. In this way, unnecessary waste of acetate strips and plasma samples may be avoided.

6. The tubes containing blood samples collected in LA-11 for use in this activity may be used to make approximate hematocrit determinations. (A hematocrit is a measure of the percentage of the volume of whole blood accounted for by erythrocytes.) If you elect this option, the hematocrit should be determined before opening the tubes and using the plasma. If you wish to have your students measure hematocrits, the following procedure should be followed. (The normal values are 36 to 46 for females and 41 to 51 for males. A low hematocrit may indicate anemia; a high value suggests polycythemia.)

a. Take the tube containing the blood sample and place it in a centrifuge tube with the end containing the settled blood cells toward the bottom.

b. Spin the blood down for about 5 minutes. (Before doing this, make sure that the silicone cement plug at the end of the tube is securely fixed to the tube.)

c. Remove the tube from the centrifuge and measure the height of the column of settled blood cells and the total height of the blood column, including the plasma fraction.

d. Calculate the hematocrit as follows:

$$\frac{\text{height of settled cells}}{\text{total height of blood column}} \times 100\%$$

7. The capillary tubes recommended for this activity should be approximately 75 to 100 mm long, 1.2 to 1.4 mm I.D., approximate volume 50-µl. These tubes may also be used in LA-16 where the students determine the coagulation time of their blood. The tubes are distributed by Scientific Products (B4197-1) and by Carolina Biological Supply (71-1040).

8. Students may be discouraged by the fact that they find only two spots while ST-13 shows five bands. You can point out that at the turn of the century, biochemists thought that there were only two blood proteins (albumin and globulin). Years later, as the techniques for separation of proteins became more sophisticated, more blood proteins were discovered. Methods that are in popular use today may be considered to be primitive in a few years.

MATERIALS: (for 10 set-ups)

10 ml acetic acid, glacial
1 g Ponceau S (MCB PX-1311)
2 g bovine albumin (Sigma A4503 or equivalent)
1 g bovine gamma globulin (Sigma BG-II or equivalent)
15 g trichloroacetic acid (Cl_3CCO_2H)
0.85 g sodium chloride (NaCl)
blood samples drawn in LA-11
10 to 20 electrophoretic (cellulose acetate) strips, Gelman 51003, SP E4710-3
20 microscope slides
10 mm rulers
20 beakers, 150-ml
10 "cut-out" Petri dishes (plastic) from LA-12
10 Petri dish covers
20 capillary tubes
10 BIP's
20 electrophoresis electrodes
paper tissues
3 dissecting pans (or similar containers for soaking acetate strips and for acetic acid baths)
10 ring stands
20 ring-stand clamps

PREPARATION OF REAGENTS:

Ionic (buffer) solution: See Preparation of Reagents for LA-12.

Saline solution: 0.85 ± 0.1 g sodium chloride (NaCl) + sufficient water to make 100 ± 1 ml solution.

Albumin standard solution: 2 ± 0.1 g bovine albumin + sufficient saline solution to make 50 ± 1 ml solution.

Gamma globulin standard solution: 1 ± 0.1 g bovine gamma globulin + sufficient saline solution to make 50 ± 1 ml solution.

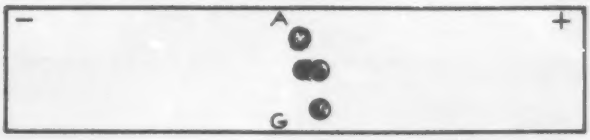
Acetic acid, 5%: 10 ± 1 ml glacial acetic acid + sufficient water to make 200 ± 5 ml solution.

Ponceau S stain, 0.5%: 15 ± 0.1 g trichloroacetic acid + sufficient water to make 200 ± 5 ml solution. Add 1 ± 0.1 g Ponceau S stain and stir to dissolve.

ANTICIPATED RESULTS:

We obtained the results as shown on the following page. Note that although the spots representing the albumin and globulin fractions of the student plasma

protein sample are fused, it is possible to see that there are indeed two different groups present. It might be possible to improve the resolution with a longer run.



ANSWERS TO DISCUSSION QUESTIONS:

1. The net charge on both the albumin and the globulins is negative.

2. The plasma proteins migrated toward the anode. There was a very slight separation of the albumin and the globulin fractions.

3. The albumin fraction is the one that migrated farther and the globulin fraction is the one nearer the point of application.

LESSON 14: IMMUNITY--AN HISTORICAL APPROACH

RATIONALE:

Activity 14 is programmed so that students will have an opportunity to rediscover some basic principles of immunity in an historical context. ST-14 extends and clarifies the concepts that are treated in the activity. The discussion of immunology will be continued through Lesson 15.

The subject of immunity is important in modern medicine. References to immunology and/or antibodies are already well entrenched in the curriculum, for example, in the discussion of hepatitis in Unit II and in the discussion of diseases that lead to changes in plasma electrophoretic patterns in this unit.

OBJECTIVES:

The student will:

- list the major function of leukocytes.
- define and describe phagocytosis.
- state where phagocytosis usually occurs in the body.
- identify the kind of leukocyte that produces antibodies.
- list at least two different ways that antibodies function to protect the body from disease.

SEQUENCE: Activity 14; ST-14

SUGGESTIONS:

1. Activity 14 requires reproduction of Chapters 2 through 6 as explained in Teaching Note 3. These materials are at the end of this lesson.

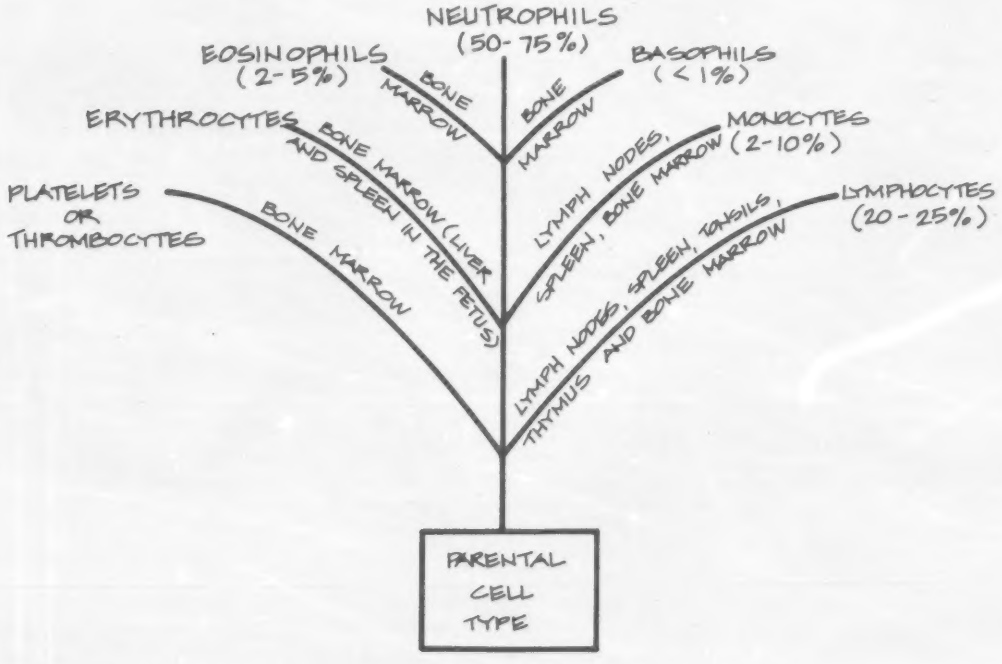
2. It is suggested that Activity 14 precede ST-14 because the activity requires students to make deductions and guesses based on classic studies in immunity --ST-14 would give away the answers. When used in the suggested sequence, ST-14 should clarify and amplify on the readings of the activity.

3. Two kinds of leukocytes (neutrophils and lymphocytes) are specifically mentioned in ST-14. The reason that they were selected is, in part, their importance in defense against infectious disease. In addition, the discussion of lymphocytes will lead to a treatment of the lymphatic system in the following lesson. You may wish to refer to the background information on leukocytes included in Lesson 11.

4. It was felt that a fuller discussion of the different kinds of leukocytes would not be essential for most high school students. Possibly one or more students might want to do some library research on the different forms of leukocytes.

One point that might be interesting to the students is that the different kinds of leukocytes are the result of a differentiation process similar to the one described for erythrocytes. Also, it is believed that all the different kinds of blood cells are derived from a single type of parental (or stem) cell.

The figure below outlines the relationship between the parental cells and the blood cells. In addition, it summarizes the proportions of different kinds of leukocytes in the blood and indicates where these cells are made.



5. Since phagocytosis is an important part of this lesson, you may wish to recycle the earlier discussion of amebae (Unit II, Lesson 34). In addition, you may wish to refer back to the discussion of hepatitis in Unit II, Lesson 9, because of the earlier discussion of antibodies.

6. The subject of antibodies and immunity is one that could be developed considerably more. Lesson 15 will extend this subject and in a later unit on genetics, we will discuss blood typing and review some concepts of immunity. Additional background information on immunology is provided for you to use as needed. This material goes well beyond the Student Text.

7. In ST-14, vaccination is defined in the way it is commonly used. The term "vaccine" comes from the Latin word for cow (vacca), since the first vaccines were derived from cows. The use of the term is no longer restricted by the source of antibodies.

8. The students may wish to discuss the ethics of Jenner's experiment on the eight-year-old boy (Section 14-4). In turn, you may wish to consider this experiment and its ethical implications with your biomedical social science colleague. There is still controversy over the ethics of experimentation on humans.

9. At the end of ST-14, students are asked to suggest a theory to explain the development of immunity in the boy in Jenner's experiment. The point to be made is that cowpox virus is much like smallpox virus. An injection of cowpox virus stimulates lymphocytes to produce antibodies against cowpox virus. These antibodies also work against smallpox virus.

10. The subject matter of Lesson 14 suggests several career possibilities. In addition, one could add hematology, cytology, histology, pathology and other health fields. You may wish to refer the students to brochures on relevant careers or consider the possibility of a field trip or speaker to supplement this lesson.

INFORMATION ON IMMUNOLOGY:

The body is constantly coming into contact with foreign "invaders" in the form of bacteria, viruses, fungi, biochemicals, etc. To combat them, there exists throughout the body a system of cells and protein molecules called the immune system (IS).

Besides invaders from outside the body there are those which originate from within the body itself: namely, cancer cells. One theory is that cancer cells are constantly developing from normal cells throughout life, and that the immune system immediately recognizes them and destroys them. According to this theory, cancer cells are free to multiply and cause disease only when the immune system doesn't recognize them as being abnormal and so fails to destroy them.

Tolerance of Self

Interestingly, the IS can cause disease as well as prevent it. The IS normally recognizes the body's own normal cells and molecules as self, and so does not destroy them--a property called tolerance of self. Some immunologists believe that tolerance of self occurs because the immune system makes contact with the body's own cells and molecules during embryonic development and learns to recognize them as self at that time. However, certain body cells don't make contact with the IS at that time, so later in adult life the IS may destroy them as if they were invaders. This may explain certain "autoimmune" diseases in which the tissues that make sperm cells and thyroid hormone are destroyed. Autoimmunity will be discussed in Lesson 15.

How the IS Fights Disease

There are two major ways the IS fights disease: humoral and cellular immunity. First we must define a key word: antigen (Ag). This is any substance that can provoke a response from the immune system. Ag's are usually proteins or complex polysaccharides; bacteria and other invaders have them in their outer surfaces. Some antigens such as bacterial toxins are not attached to a cell.

The bacterium that causes tetanus, Clostridium tetani, will be our example of humoral immunity. This organism gives off a toxic protein which spreads in the bloodstream to affect nerves. The toxin acts as an antigen. It provokes the lymphocytes in lymph nodes and elsewhere into making proteins called antibodies (Ab's). These Ab's flow in the blood plasma and other body fluids and hence are called humoral Ab's. This kind of immune response is thus called humoral immunity. The Ab's bind to the Ag, in this case a toxin, which renders the Ag harmless.

The Ab's made are specific for the tetanus Ag and no other. Ab's bind onto the Ag they are specific for, forming an Ag-Ab complex. The Ag-Ab bond is not a covalent bond, but one of much weaker forces that exist between atoms. Only a small portion of the antibody molecule binds to the antigen molecule; it has been estimated that the binding zone contains about six amino acids. The "bond" is thought to involve several points of binding and both polar and ionic attractions. Because the binding is very specific, much like an enzyme-substrate complex, the antigen-antibody complex is often compared to a lock-and-key relation. A specific antigen fits a specific antibody.

Now we will examine a different kind of immune response, cellular immunity. If a tuberculosis bacterium enters the body, some of the lymphocytes that come into contact with the bacterium possess Ab's against the bacterial Ag. These Ab's are bound onto the outside of a lymphocyte's cell membrane. There are about 100,000 such Ab molecules on each lymphocyte. Such lymphocytes are called sensitized lymphocytes.

When the Ag binds to the Ab's on sensitized lymphocytes, these lymphocytes transform; they grow larger and secrete substances which cause neutrophils and other white blood cells to collect in the area and phagocytize the TB bacteria. How

How sensitized lymphocytes arise is a mystery. It is known, though, that vaccination and exposure to the disease organism makes their numbers increase.

Cellular immunity is the main defense against TB, fungi, protozoa, worms, cancer cells, poison oak, transplanted organs (unfortunately) and some viruses (influenza, smallpox). Some diseases stimulate cellular immunity to work (smallpox), others humoral immunity (polio, diphtheria). Notice that both kinds of immune response involve lymphocytes. The lymphocytes of humoral immunity, as well as those of cellular immunity, have Ab's bound to their membranes, waiting for the appropriate Ag to come along. When one does, the humoral lymphocytes proceed to make humoral Ab's. The lymphocytes of cellular immunity, on the other hand, make substances that encourage cells to collect around the intruder and phagocytize it.

The lymphocytes of humoral and cellular immunity come from two different places. Humoral lymphocytes are derived from the bone marrow, lymph nodes, spleen and lymphocyte-rich areas in the intestine and tonsils. The lymphocytes of cellular immunity, in contrast, come from the thymus gland in the chest. Both kinds make up the white blood cells we call lymphocytes in blood, and have the same appearance.

How exactly do the lymphocytes of humoral immunity make humoral Ab's? The first time a specific Ag enters the body, certain cells (macrophages) phagocytize the Ag or the cell that contains it and then wander until they end up in the lymph nodes. It is inside these organs, which are located throughout the body along the lymphatic vessels, that lymphocytes contact the Ag that the macrophages have brought. By an unknown process, this provokes the lymphocytes to grow large and multiply and manufacture Ab's that enter body fluids (humoral Ab's).

On second exposure to an Ag all this occurs much faster, for certain macrophages and lymphocytes are thought to carry a memory of the first Ag, and as soon as they contact the Ag they go to work. Their memory consists of the Ab's on their surface which are specific for an Ag, similar to the sensitized lymphocytes of cellular immunity.

Complement: How It Helps Humoral Ab's Work

We will now examine what happens after the Ag-Ab complex forms. One thing the Ag-Ab complex does is cause reactions among certain proteins called complement found in the body fluids. When the Ag's on bacterial surfaces bind Ab's, the complement proteins react with the Ab's to make holes in the bacterial cell membrane, killing the cell. Complement reaction products also:

1. attract neutrophiles into the area to phagocytize the bacteria, and

2. cause neutrophils and special cells called mast cells to release histamine, a chemical that causes fluid to collect in the area. This helps explain the swelling seen in infected tissue. It is another example of edema.

Complement doesn't affect all bacteria, nor does it affect all viruses or toxins. Ab's have the following other methods of combating invaders.

1. Ab's clump bacteria, a process called agglutination. The bacteria clump because each Ab has more than one Ag-binding site. Clumps are then easy targets for phagocytic cells to destroy.

2. Before a virus can damage a body cell, it must attach to the outside of the cell. Ab's bind to the attachment point of the virus and so prevent the virus from attaching. This is called virus neutralization.

3. Antitoxins are antibodies against toxins. They are described for tetanus above. They are also used against botulism and anthrax toxins.

INFORMATION ON ACTIVITY 14:

TEACHING NOTES:

1. The purpose of this activity is to provide some background in immunology in a format that will require students to analyze scientific observations.

2. Anticipated time: one to two periods.

3. Chapters 2 through 6 of the activity in this lesson were not provided directly to the students because they contain the answers to problems the students are asked to consider in earlier chapters. It is desirable to make at least one copy of Chapters 2 to 6 per two students. These chapters are provided after these teaching notes. The answers are given at the end of this lesson.

4. This activity is designed for student groups, with an ideal group size of three to four students. (Individual students or pairs of students may have difficulty coming up with the desired solutions. Also, if the groups are too small, you will have too many groups to handle.)

After the students read the Introduction and Chapter 1 (in the Laboratory Manual), have each group discuss, write an answer to the question at the end of Chapter 1 and give it to you for evaluation. When you feel that they have thought through the question sufficiently and responded adequately, given them Chapter 2 to read. It is not crucial for the student groups to produce all the correct answers; the main point is for the students to read and try to use the material. After the students complete Chapter 2, have them hand in their answers, and if their answers are adequate, have them proceed chapter by chapter until they complete the assignment. (If their answers are wrong, you can provide a clue and ask them to try again.)

5. You may wish to provide some laboratory materials to break up the long reading assignments and to add interest to the activity. You could provide a demonstration slide of starfish larvae to go with Chapters 1 and 2. A vial of preserved starfish bipinnaria may be obtained from biological supply houses (for example, Carolina Biological #P341).

The starfish larvae will be at various stages of development. Try to find one similar to the illustration in Chapter 2 below.

6. To demonstrate phagocytosis, you could provide the students with a culture of Ameba proteus. The amebae are usually a little hard for students to locate, so if you use this option the students may need help. Amebae are generally found near the bottom of a culture. Cultures of amebae often contain a high proportion of ciliated protozoans--point out to students that these fast moving protozoans are not amebae but are added as food for amebae.

Sprinkle a little powdered charcoal on the ameba slide to see whether the amebae phagocytize the particles. It will take some time and patience to locate an ameba in action.

CHAPTER 2

Metchnikoff focused on a starfish larva and then went back to the garden. From the roses he broke several thorns to use as "harmful intruders." At his microscope again, he inserted the rose thorns into several larvae. And then he waited--impatiently.

"Very nervous, I did not sleep during the night, as I was waiting for the results of my experiment," he reported. "The next morning, very early, I found with joy that my experiment had been most successful."

As he had guessed might happen, the microscope revealed that each rose thorn tip inside a larva was surrounded by a cluster of the wandering cells that he had watched the day before.

STARFISH LARVA

This little experiment was the basis for a theory of immunity that was to hold Metchnikoff's attention for 25 years. From this and further experiments, he decided that certain cells were the major defense against bacterial infection in all animals. He labeled such cells "phagocytes," which means "eating cells." He suspected that they protected the body by surrounding and digesting invading bacteria.

Of course, Metchnikoff could hardly rest his case on observations of rose thorns in starfish larvae. The experiment had no direct bearing on bacterial infection. When Metchnikoff returned home to Russia, he decided to study a more natural situation--one more related to microbes and disease. Again, he chose an animal that would be easy to observe--this time the transparent water "flea," Daphnia, like the one you observed in Laboratory Activity 7.

The disease that Metchnikoff studied was one caused by certain fungus cells that Daphnia may swallow. Once swallowed, the cells can enter the intestines and the body cavity. Unchecked, they then grow and multiply, bringing death to the Daphnia.

But Metchnikoff found there was a check, provided there weren't too many fungus cells. Each cell that entered the body cavity of a Daphnia was soon surrounded by several ameba-like cells. Then slowly these cells would digest and destroy the invader. These results were pleasing to Metchnikoff because they were similar to the results in the starfish experiment. Yet Metchnikoff's early research into phagocytosis seems rather far removed from human beings and human disease. Starfish and Daphnia are animals, certainly. But they are very different from man. Metchnikoff knew that phagocytosis occurred in simple animals but did not know whether it could occur in humans. So he decided to look into a disease of humans: anthrax.

The German bacteriologist Robert Koch had shown that anthrax is caused by bacteria. Louis Pasteur had developed a vaccine that made an animal immune to this fatal disease.* It was also known that a person could be made immune by exposure to very small amounts of anthrax bacteria.

Pasteur's feat of vaccination against anthrax was well known to Metchnikoff. Metchnikoff believed that he could explain the immunity in terms of his own theory about phagocytic cells.

Question: If Metchnikoff's phagocytic theory is correct, how can the beneficial effect of vaccination be explained?

* The term "vaccine" comes from the Latin word for "cow." It is now used for preparations of pathogens which have been changed so that they no longer cause disease. At Pasteur's time, the term referred to preparations taken from cows. Such preparations contained microbes.

CHAPTER 3

Metchnikoff attempted to discover how a vaccinated animal resisted the anthrax bacteria. He compared the action of blood from a vaccinated animal and from a non-vaccinated animal on the anthrax bacteria. In some experiments, he introduced the pathogen under the skin on the ear of a rabbit. Then he would repeatedly draw off the pus that formed to observe the leukocytes. He observed phagocytosis in the blood of both vaccinated and non-vaccinated rabbits, but the phagocytosis was much more active when the blood from a vaccinated animal was added to anthrax bacteria. Vaccination had apparently stimulated the activity of some kinds of leukocytes--they could be seen to move faster and destroy anthrax bacteria by phagocytosis.

But Metchnikoff's conclusions were not shared by all scientists in the field. Other experiments suggested that immunity might depend on some compound in the blood plasma rather than on the white blood cells. In fact, some scientists suspected that even Metchnikoff's observations on the increased phagocytosis were not really related to immunity.

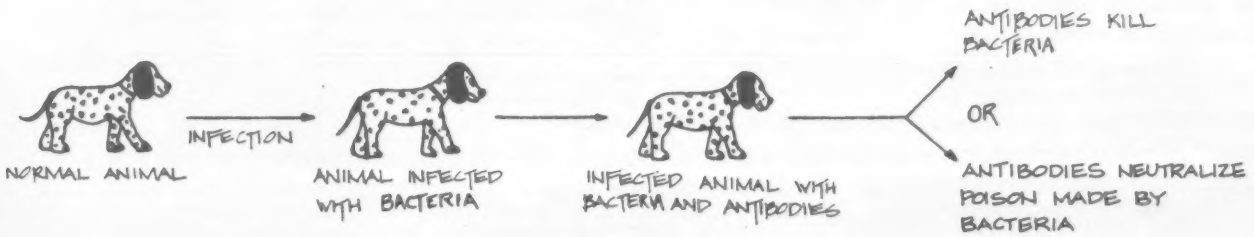
These hints of a different mechanism of immunity led a physician named George Nuttall to repeat some of Metchnikoff's experiments. Nuttall observed, just as Metchnikoff had, that some leukocytes from the blood of various vaccinated animals did capture anthrax bacteria; that is, they acted as phagocytes. But he saw something else which Metchnikoff hadn't reported. Many anthrax bacteria died even where there were no leukocytes. Nuttall concluded that immunity could be explained without reference to phagocytes. He believed that the phagocytes did not really contribute to the immunity.

Question: How is it possible to explain immunity without reference to phagocytes?

Experiments such as Nuttall's convinced Nuttall and some other scientists that phagocytes really have no important role in immunity. Some chemical substance in the blood plasma--some antibody, as such substances came to be called--kills the invading bacteria. Then the phagocytes merely clear away the debris rather than kill the bacteria. Two different theories were thus current.

Metchnikoff--Phagocytic white blood cells surround and destroy invading bacteria.
Nuttall--Plasma factor (antibody) kills bacteria.

One scientist who was basically in agreement with Nuttall was a German, Emil von Behring. But von Behring saw two different ways that blood plasma might given immunity to bacterial attack. One was the way Nuttall had suggested--that antibodies in the blood plasma kill the bacteria. But he also suggested another possible explanation--that antibodies might cancel the effects of the bacteria and not necessarily kill the bacteria. For example, the antibody could react with a bacterial toxin and neutralize that poison.



When von Behring raised this question about the two different ways in which antibodies might function, it was known that some bacteria cause disease by making toxins that spread through the body. Diphtheria had been found to be such a disease. The bacteria that cause diphtheria lodge in the throat and then slowly but steadily produce a toxin. This poison may eventually kill the host, unless the body has some defense against it.

Emil von Behring discovered that the defense that the body may develop against such bacteria is an antibody that neutralizes the toxin. For example, the defense of the body against diphtheria is to generate an antibody to counteract the poison produced by the bacteria.

But what of the bacteria themselves? Once lodged in the body, would they go on producing toxin forever, perhaps multiplying as time went on? Or might they eventually fall victims of Elie Metchnikoff's phagocytes?

Von Behring apparently didn't answer that question. But it is doubtful that he would have admitted that phagocytes could play so important a role. At that time, scientists still suspected that immunity could be explained by a single mechanism--either one involving antibodies or one involving phagocytes. Metchnikoff still believed that the answer lay wholly with the phagocytes. Von Behring saw instead the antibodies in blood plasma as being the whole answer. And other scientists generally lined up behind one man or the other.

Von Behring's reaction to Nuttall's experiments had been to decide that antibodies in blood plasma were responsible for immunity. But later scientists saw in the same experiments hints of another possible explanation. Possibly immunity was neither one thing nor the other, but both. Perhaps antibodies and phagocytes worked together to ward off bacterial invasions.

Question: How might such a cooperative effort occur?

CHAPTER 5

By 1903 it was becoming clear that neither blood plasma nor phagocytes alone could explain all the facts of immunity. It was becoming clear that the role of phagocytes must be more than sweeping up the debris. The research of such scientists as Nuttall and von Behring had not really eliminated phagocytes as participants in immunity.

An English scientist named Almroth Wright wondered whether immunity might not be a two-step process involving both antibodies and phagocytes. His idea was that the important role of the antibodies might be to prepare the way for the phagocytes. He guessed that an antibody in the blood might be able to alter a bacterium in such a way that a phagocyte could more readily engulf it. Wright gave such hypothetical antibodies the name "opsonins," from the Latin word for "relish." They seemed to make bacteria "tasty" to the phagocytes. He then set about showing that opsonins exist.

Two of Wright's famous experiments are summarized in the table below. In the first experiment, he added blood plasma to Staphylococci and allowed some time for the opsonin to work on the bacteria. Then he mixed in some phagocytes, and after another time interval counted the number of bacteria inside the various phagocytes. From his count he arrived at an average consumption of 30 Staphylococci per phagocyte.

This experiment by itself does not prove that the blood plasma contributes to phagocytosis, so Wright performed a second experiment, but with one difference. Before mixing the Staphylococci and the blood plasma, he heated the plasma to 60 to 65 °C for 10 to 15 minutes, and then cooled it back to the proper temperature. That little cooking operation was intended to destroy the opsonin that he felt must be there. And it appeared that the cooking had at least greatly reduced the effectiveness of the opsonin. Note that when the phagocytes were then mixed in, they consumed a mere four bacteria on the average instead of the previous 30.

Experiment	Ingredients		Average Consumption of Staphylococci per Phagocyte
	Part A	Part B	
1	Staphylococci + Blood Plasma	Ingredients of Part A + Phagocytes	30
2	Staphylococci + Heated Blood Plasma	Ingredients of Part A + Phagocytes	4

These results encouraged Wright in his hypothesis that opsonins alter bacteria making them easier for the phagocytes to destroy, but he recognized that his experiments failed to prove it. The blood plasma might have had no effect on the bacteria at all. The heat may have done something else.

Question: What other interpretation of Wright's experiment (besides opsonin action) is possible?

CHAPTER 6

It probably wasn't fair, but a fellow scientist once referred to Sir Almroth Wright as "Sir Almost Right." Opsonins seemed real to Wright, but he could never quite prove they were there. First, if there were opsonins or some other substance in the plasma, how could he be sure they didn't work by affecting the phagocytes instead of the bacteria? In other words, the opsonin or some other substance in the blood might simply stimulate the phagocytes to eat hearty.

Wright did a number of experiments that were aimed at answering that question, but none succeeded. Eventually, though, other scientists were able to show that the antibodies that increase the consumption of the phagocytes do so by acting on the bacteria rather than on the phagocytes. These antibodies are opsonins.

As more and more scientists contributed to a theory of immunity, it began to appear that maybe everybody was partly correct. Wright's work added to that of von Behring and Nuttall and Metchnikoff--and many others--began to make it evident that immunity is not a simple phenomenon. The body, it turns out, has a mixed bag of tricks. Some bacterial diseases are fought by plasma antibodies that kill the bacteria directly or neutralize bacterial toxins. In others, the plasma antibodies (opsonins) merely prepare the bacteria for their destruction by the phagocytes. And in some, the phagocytes are the main line of defense.

SOME FINAL THOUGHTS:

In these six chapters, you have seen how some of the basic concepts of immunity were discovered and how these concepts evolved during the last century. You have seen something of the complexity of scientific research and how research is stimulated by controversy and curiosity. Finally, and most important, you have been asked to play the role of a scientist and to anticipate some of the great experiments in the development of our current understanding of immunity. This is not an easy thing to do; but it is challenging and, for the experimentally inclined, fun. This activity provides you with an inkling of what scientific research is like. Laboratory research offers opportunities for many careers. These careers generally require a background involving college and graduate-school degrees. A research career may appeal to the individual who likes intellectual challenge. There is also the chance to make a major contribution to medicine.

ANSWERS TO CHAPTER QUESTIONS:

1. To help answer his question about the role of migrating cells within starfish larvae, Metchnikoff introduced a "harmful intruder" into the larvae. He used rose thorns as the "intruder." This experiment was performed to see whether the behavior of the migrating cells would change if an intruder was introduced--whether these cells would move toward the thorn.

2. If Metchnikoff's phagocytic theory is correct, the beneficial effect of vaccination can be explained by the phagocytic cells of the body becoming more efficient or more numerous as a result of the vaccination.

3. Nuttall observed that anthrax bacteria died without being phagocytized--something from the blood plasma killed the invading bacteria.

4. Antibodies and phagocytes could work together in a cooperative effort to ward off bacterial invasions. Antibodies could change the bacteria, making it easier for phagocytes to destroy them. Another possibility is that antibodies could stimulate the phagocytes to become more active. A third possibility is that antibodies and phagocytes could each kill some of the bacteria.

5. Other interpretations of Wright's experiment (besides opsonic action) are possible. The blood plasma is the medium in which the phagocytes live. Heating the blood plasma may have altered the plasma, making it difficult for the phagocytes to function. It may also have affected the bacteria, making them somehow less appetizing to the phagocytes.

LESSON 15: IMMUNITY AND THE LYMPHATIC SYSTEM

RATIONALE:

This lesson extends the brief treatment of immunity in Lesson 14. In addition, it supplements earlier lessons on other topics and ties them to immunity. For example, botulism (a form of food poisoning discussed in Unit II) is reconsidered in relation to active and passive immunity. Also, electrophoretic patterns are reconsidered in view of the fact that most antibodies are gamma globulins. The lymphatic system is then described since it is the source of much of the body's lymphocytes. Finally, the lymphatic system is shown to help in prevention of edema. LA-15 provides a simulation of antigen-antibody reactions in a clinical context.

OBJECTIVES:

The student will:

- distinguish between active and passive immunity.
- list one advantage and one disadvantage of passive immunity.
- state the significance of electrophoretic patterns showing high or low concentrations of gamma globulins.
- list at least two functions of the lymphatic system.
- explain how the lymphatic system helps to prevent edema.
- determine the titer of a simulated antibody solution.

SEQUENCE: LA-15; ST-15

SUGGESTIONS:

1. We suggest doing LA-15 before ST-15 merely to break a rather long reading assignment. (Both Activity 14 and ST-14 involve reading.)

2. In Sections 15-1 and 15-2, rectangles are used to encompass review material in order to provide the students with a convenient reference.

3. You may wish to check whether students understand that "passive" is the opposite of "active." Otherwise they could miss the point about the difference between active and passive immunity.

4. Botulism is referred to in Section 15-1 as an example of a toxin which requires a quick source of antibodies (i.e., passive immunity). The toxin produced by Clostridium botulinum was discussed in Unit II in relation to food processing (ST-37). Some background information on botulism follows the list of suggestions--you may wish to use some of this information to remind students of the relation between Units II and III.

5. Another tie between Units II and III that merits emphasis is the analogy between the specificity of antibodies and the specificity of enzymes (Unit III, Section 15-2 and Unit II, Sections 4-4 and 17-3). Specificity in both cases depends on the three-dimensional structure of proteins which, in turn, is a function of the amino acid composition of proteins.

6. Section 15-2 relates the fact that most antibodies are gamma globulins to some clinical applications of electrophoresis (ST-13). You might review Problem Set 13 with reference to this new piece of information.

7. The lymphatic system is of importance in human physiology for the reasons explained in this lesson. This system might have been introduced conveniently in the Nutrition Unit in relation to transport of fats or in this unit in relation to prevention of edema. We chose, however, not to introduce the lymphatic system until we had established a background for considering the major functions of the lymphatic system. Lesson 15 may be used to review fat transport and prevention of edema and to relate both topics to the lymphatic system.

8. The role of the lymphatic system in relation to absorption and transport of lipids has been simplified in the text. A more complete explanation follows. Recall that absorption of fats occurs in the small intestine and that fats enter the small intestine primarily as triglycerides (esters of glycerol and three fatty acids). The triglyceride is partly digested by lipase to form fatty acids and a monoglyceride. These fatty acids and monoglycerides are absorbed, but they are not all transported by the lymphatic system. The smaller fatty acids with less than a dozen carbons tend to enter the portal vein for transportation to the liver. However, most fatty acids absorbed are larger. These longer-chain fatty acids are metabolically converted back to triglycerides, which are then coated with proteins to form minute droplets. The droplets then enter the lymphatic system instead of the portal vein. They bypass the liver and enter the bloodstream via the thoracic duct and are then distributed throughout the body.

9. The lymph nodes have another function besides producing lymphocytes. They act to strain and purify the lymph prior to its return to the bloodstream. The lymph nodes contain large phagocytic cells (macrophages) that remove and destroy dead cells, bacteria and other kinds of debris.

The greatest concentrations of lymph nodes are in the neck, the armpit, the bend of the arm and the groin. Swelling of specific nodes occurs when there is a localized infection because dead cells and interstitial fluid drain into the lymphatic system. Generalized enlargement of the nodes occurs in infectious mononucleosis and certain other systemic conditions.

10. Students may be aware that there is a relationship between immunology and allergies. You may wish to point out that antibody production can actually be harmful, as in allergies and autoimmune diseases. The concept of an allergic response was touched on in the Respiration Unit (ST-27). Additional background information on allergies and autoimmunity follows.

INFORMATION ON BOTULISM:

Botulism was first recognized as a disease more than two centuries ago. German

physicians were aware that people were getting sick and dying as a result of eating spoiled sausages. They called the condition "botulismus" from the Latin word for sausage (botulus).

The causative pathogen is Clostridium botulinum. It is sometimes difficult to destroy this pathogen during food processing for two reasons. First this organism is anaerobic. While many pathogens cannot survive inside a can of food because of the absence of oxygen, Clostridium grows best in the absence of oxygen. Secondly, Clostridium cells can form spores. In the spore form, this microbe is especially resistant to destruction by heat. Such spores can resist 100 °C heat for several hours. They are, however, destroyed by moist heat at 120 °C in an autoclave.

The disease is usually traceable to home-canned foods, rather than commercially processed foods. This is because of the sterilization problem discussed above. In the U.S., home-canned fruits and vegetables are the prime sources.

There are actually several different strains of Clostridium botulinum, designated by capital letters "A" through "F." They produce toxins that can kill both humans and other mammals. Most human cases and most human fatalities are caused by toxins of strains A and E.

The botulinum toxins are among the most deadly poisons known. Two of these toxins have been highly purified and shown to be proteins that are absorbed primarily in the stomach and the small intestine.

The toxin blocks transmission of nerve impulses. Early symptoms include weakness, dizziness and nausea. If the disease is diagnosed this early (usually within a few days after ingestion of the toxin), the victims can be helped by injections of antibodies. However, if the toxin has attacked the nervous system, then the symptoms are more severe and the therapy more drastic. Neurological symptoms may include blurred vision, speech difficulties, palsy and muscle weakness. The main cause of death is respiratory paralysis and secondary pulmonary infection. Sometimes patients in late stages of the disease may be saved by surgery and/or use of a respirator.

INFORMATION ON ALLERGIES AND AUTOIMMUNE DISEASES:

An allergy is defined as an acquired specific alteration in an organism's biological reactivity due to exposure to an allergen. An allergen is any substance that initiates this alteration. Allergens range from pollen and cat fur to chemicals contained in foods. Allergies are further characterized by the fact that the alteration in reactivity of the organism is undesirable. In other words, the immune system reacts in such a way as to create disease within the body rather than prevent it. Allergic responses involve one particular class of antibody known as immunoglobulin E. This protein is present in the body in very small quantities and located on the membranes of cells such as neutrophils and basophils. When the antigen to which a person is allergic comes in contact with cells containing immunoglobulin E, the release of histamine is triggered.

Histamine causes fluid to accumulate wherever it is released. This explains local skin swelling as in hives. Histamine also stimulates mucus glands, which explains hay fever symptoms such as running nose and teary eyes. Antihistamines are thus helpful in treating allergies.

Histamine and other substances released can constrict the bronchi in the lungs, causing breathing difficulty, as in an attack of allergic asthma. Such bronchial constriction is sometimes sudden and accompanied by a severe drop in blood pressure; it is then called anaphylaxis or anaphylactic shock and can be fatal. Some people go into anaphylactic shock after a bee sting or inhaling ragweed pollen or getting a shot of penicillin.

Some allergies can be treated by injecting very small amounts of the suspected antigen repeatedly over a time. This stimulates the production of certain antibodies such as immunoglobulins A and B. These immunoglobulins work differently than immunoglobulin E. Rather than causing release of histamine, they bind to the offending antigen before the immunoglobulin E reaches the antigen. This prevents the allergic response. Such treatment is called desensitization.

In some diseases, the immune system actually destroys the body's own proteins or cells. Such conditions are called autoimmune diseases, because the body acts as if its own cells were undesirable invaders. Although the study of autoimmune diseases is relatively new, there is already an impressive number of diseases that fit into this category. This list includes thyroiditis and rheumatoid arthritis.

Consider rheumatoid arthritis as an example of an autoimmune disease. Though it is not usually fatal, its victims are slowly disabled over many years. More than two million Americans are thought to have this disease. Most people with rheumatoid arthritis contain a certain chemical in their blood called the rheumatoid factor. This factor has been shown to be an antibody that attacks the patient's own immunoglobulins. In other words, it is an antibody against antibodies! Precisely how this factor is related to the degenerative process that characterizes rheumatoid arthritis is not yet known.

INFORMATION ON LABORATORY ACTIVITY 15:

TEACHING NOTES:

1. The purpose of this activity is three-fold: (1) to introduce the students to a clinical technique commonly used to determine the antibody levels in serum; (2) to point out some of the clinical applications of this technique; and (3) to provide additional information on antibody-antigen reactions to supplement the material in the Student Text.

2. Anticipated time: 1 period.

3. Simulated antigens and antibodies are used because of the high cost of antiserum (serum containing antibodies to a specific antigen). However, blood typing will be done in a later unit, and this involves "real" antisera and antigens. The simulated antigen and serum samples in this activity are Na_2CO_3 and $BaCl_2$, respectively. The chemical reaction responsible for the production of the white precipitate is

$$\underset{\text{(antigen)}}{Na_2CO_3} + \underset{\text{(antibody)}}{BaCl_2} \rightarrow 2\,NaCl + \underset{\text{(antibody-antigen complex)}}{BaCO_3\downarrow}$$

The students should be familiar with this reaction from Unit I, LA-26, where it was the basis for their study of chemical reactions.

4. The titers obtained in this simulation and referred to in the discussion questions are not based on typical hepatitis antibody concentrations. They have been chosen to correspond to dilutions that are easy for the students to make.

5. All of the solutions described under Preparation of Reagents have been designed to give a concentration of 0.001 M $BaCl_2$ at one of the dilutions specified in the procedure (see Anticipated Results). At this concentration, there should be no visible precipitate and this value will provide the titer. However, the dilutions that the students make to reach this concentration will vary, and therefore the titers will vary as well.

6. You may wish to add a drop or two of yellow food coloring to the simulated samples since serum is typically a yellowish color. This also makes the layering more dramatic.

7. It might be a good idea to demonstrate the technique of layering before the students begin the activity. It is very important to release the "serum" from the pipet very slowly to avoid mixing of layers.

8. Note that the Materials List specifies rather large quantities of test tubes and pipets. Other glassware might be used for dilution of the serum, but keep in mind that at least four test tubes are needed for each serum sample tested. Also, if there are not enough 1-ml pipets, they may be rinsed thoroughly and reused.

9. Another technique used to determine whether or not antibodies to a specific antigen are present in an individual's serum is the immunodiffusion or Ouchterlony plate method. If you would like to demonstrate this technique to the class, it can be done with the simulated serum and antigen samples used in this activity as follows.

a. Prepare a Petri dish with plain (not nutrient) agar as shown in Figure 1. (The agar is a 2% solution.) The dish should be filled to a depth of about 5 millimeters.

When the agar has solidified, five holes should be made as indicated in the figure. The four outside holes in the agar should all be equidistant from the center and from each other. Figure 1 may be used as a template for positioning the holes. The holes can be made using a cork borer and should be about 5 mm in diameter.

b. Place about 0.1 ml (3 drops) of simulated antigen in the center hole and 0.1 ml (3 drops) of simulated serum in each of the outer holes.

c. Let the plate sit overnight.

d. If antibodies are present, precipitate patterns will form similar to the ones shown in Figure 2. If serum samples were used containing different amounts of antibodies, the precipitation lines will be thicker or thinner depending upon the concentration.

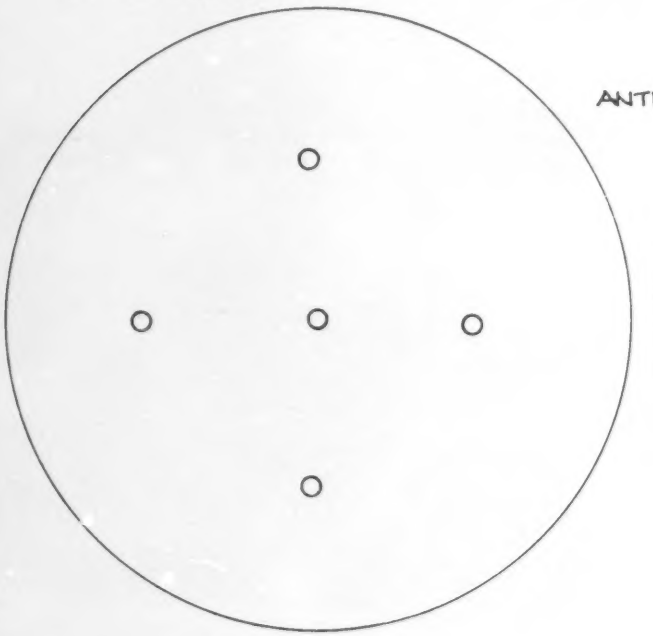


FIGURE 1: Preparation of agar plate.

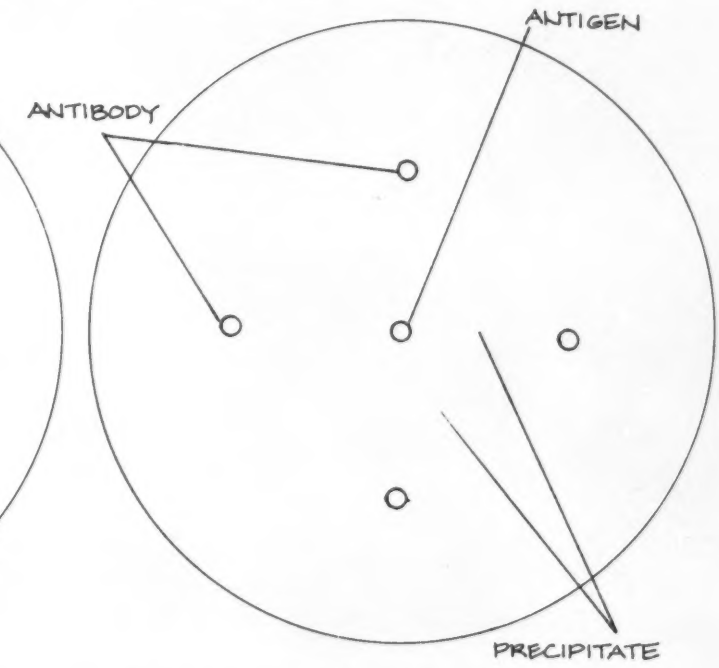


FIGURE 2: Results of immunodiffusion.

MATERIALS: (for 15 set-ups)

21.2 g anhydrous sodium carbonate (Na_2CO_3)
24.4 g barium chloride ($BaCl_2 \cdot 2H_2O$)
yellow food coloring (optional)
135 test tubes, 13 x 100 mm
15 graduated cylinders, 10-ml
45 rubber stoppers, #00
15 pipets, 10-ml
90 pipets, 1-ml
15 test-tube racks
15 glass-marking pencils
Petri dish with agar (optional)

PREPARATION OF REAGENTS:

Simulated antigen sample (1.0 M Na_2CO_3, 200 ml): 21.2 ± 0.1 g Na_2CO_3 + sufficient water to make 200 ± 2 ml solution.

Simulated serum samples (1.0 M, 0.1 M and 0.01 M $BaCl_2$): 24.4 ± 0.1 g $BaCl_2$ + sufficient water to make 100 ± 1 ml solution. Dilute 10 ± 0.1 ml of this 1 M solution to 100 ± 1 ml with water to give a 0.1 M $BaCl_2$ solution. Dilute 10 ± 0.1 ml of the 0.1 M solution to 100 ± 1 ml with water to yield the 0.01 M $BaCl_2$ solution. Add yellow food coloring if desired. (These solutions should be coded with letters known to you but not to the students. You might also use a plain water sample so that a titer of zero would result in some cases.)

ANTICIPATED RESULTS:

When the serum sample is diluted to a concentration of 0.001 M $BaCl_2$ the test yields a negative result. The dilution that is necessary to reach this 0.001 M concentration varies depending upon the concentration of the initial sample.

Unknown (Serum sample with "antibodies")	Dilution Factor	Antibody ($BaCl_2$) Concentration [M]	Antibody-Antigen Reaction (+ or -)	Titer
A (1.0 M $BaCl_2$)	0	1	+	
	10	.1	+	
	100	.01	+	
	1000	.001	-	1000
B (0.1 M $BaCl_2$)	0	.1	+	
	10	.01	+	
	100	.001	-	100
	1000	.0001 (10^{-4})	-	
C (0.01 M $BaCl_2$)	0	.01	+	
	10	.001	-	10
	100	.0001 (10^{-4})	-	
	1000	.00001 (10^{-5})	-	
D (water)	0	0	-	0
	10	0	-	
	100	0	-	
	1000	0	-	

ANSWERS TO DISCUSSION QUESTIONS:

1. See Anticipated Results.

2. Only students whose serum samples gave a titer of zero should answer this question positively.

3. If a patient has a set of symptoms so general that a number of infectious diseases may be possible, a physician can often rule out some diseases by having a laboratory technologist perform an antibody determination on the patient's serum. If the person's titer for antibody X is zero, then presumably he is not suffering from the disease caused by the antigen for which antibody X is specific.

4. Determine the greatest dilution that gave a positive result. Prepare a series of dilutions filling the gap between this value and the next dilution factor. For example, if a dilution of 100 gave a positive test and a dilution of 1000 gave a negative test, one could test dilutions of 200, 300, etc.

5. A titer of 10,000 means that the serum sample had to be diluted 10,000-fold to obtain a negative reaction, while a titer of 100 means that the sample had to be diluted 100-fold. This implies that the serum with a titer of 10,000 has a 100

times greater concentration of antibodies than the serum with a titer of 100 (10,000 = 100 x 100).

LESSON 16: BLEEDING AND CLOTTING

RATIONALE:

The processes of blood clotting, blood vessel constriction and platelet aggregation are all essential to the healing of wounds. ST-16 focuses on blood clotting. The relationship between blood clotting and hemophilia is shown with a case history involving the royal family of Russia in the early twentieth century. This particular case history will be reconsidered in a later unit as an example of sex-linked inheritance. In LA-16 the students study blood clotting with a microscope and determine the time it takes for their blood to clot.

OBJECTIVES:

The student will:

- state the relationship of hemophilia to blood clotting.
- list three processes that contribute to stopping blood flow from a wound.
- define platelet and state why platelets are important in stopping bleeding.
- state the roles of fibrin and thrombin in blood clotting.
- describe the microscopic appearance of a blood clot.
- measure the coagulation time of a blood sample.

SEQUENCE: ST-16; LA-16

SUGGESTIONS:

1. ST-16 begins with a case history about a famous hemophiliac--Alexis, the son of Czar Nicholas II and Czarina Alexandra. The story is one that will be reconsidered from the standpoint of human genetics in a later unit.

The relationship between Rasputin and the royal family may be of interest to the students and is alluded to in ST-16. Some historians believe that Rasputin was one of the major factors in precipitating the Russian revolution. This is a subject the students may wish to discuss after reading ST-16. Perhaps your social science colleague can give you some support on this subject. He should be forewarned that questions about Rasputin and the royal family may be raised in class.

2. The term "hemostasis" means the "stoppage of a flow of blood." "Hemostasis" might be a good title for ST-16 since it describes the entire section. However, the term was deliberately not used for two reasons. First, the term is not essential to later lessons in this curriculum. Second, "hemostasis" is easily confused with "homeostasis" and the latter term will be used often.

3. ST-16 discusses the platelets. Platelets are sometimes classed with the leukocytes. However, they are actually cell fragments rather than cells. They arise by breaking off from megakaryocytes, large bone-marrow cells. Platelets are much smaller than red blood cells. Like erythrocytes, the platelets lack a nucleus. They average about 200,000 to 300,000 per mm^3 of blood. This compares to concentrations of about 3,000 to 10,000 per mm^3 for leukocytes and 4 to 5 million per mm^3 for erythrocytes.

4. As indicated in ST-16, the process of blood clotting is very complex and is not yet completely understood. We chose to stress the final stages of the process

because they make the point about the chemical basis of clotting and because the final steps relate directly to the blood clot.

Actually, there appear to be two partially separate systems for blood clotting --an intrinsic system and an extrinsic system. One of the two systems involves only factors present within plasma and is therefore called "intrinsic." The other system includes a tissue factor in addition to plasma factors and is therefore known as the "extrinsic" clotting pathway. Background information on hemostasis is provided and may prove useful if you wish to expand on the clotting process.

5. An interesting question to consider that is only touched on in ST-16 is "Why doesn't circulating blood clot normally?" If the question is not raised by students, you may wish to bring it up for discussion.

Answer: The clotting process may be viewed here as an example of homeostasis. There are factors that favor blood clotting as we have discussed, and there are factors that oppose blood clotting. The blood clotting mechanisms are normally "turned off" in circulating blood. For clotting to occur, the process must be activated. Activation is triggered by blood coming into contact with an injured surface or air and involves the release of substances that help convert prothrombin to thrombin. An additional safeguard is a circulating plasma polysaccharide, heparin, which inhibits the conversion of prothrombin to thrombin. In spite of all of these homeostatic regulators, clotting sometimes does occur in circulating blood and is an important medical problem.

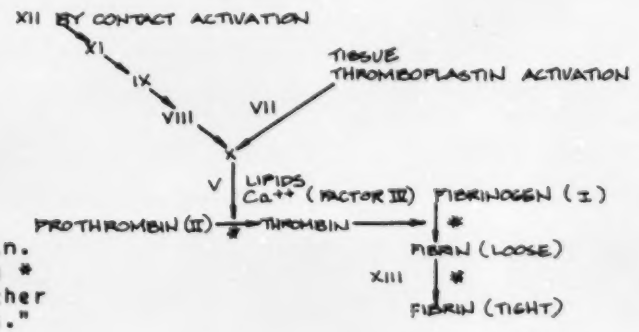
6. It may be a good idea to review atherosclerosis and CHD after discussion of blood clotting. (See Student Text, Section 9-2.)

INFORMATION ON HEMOSTASIS:

Microscopic blood vessels are constantly tearing open inside the body (microhemorrhaging). The body has a mechanism to seal off blood leaks from internal bleeding and injuries from outside the body. The mechanism is called hemostasis.

Damage to a blood vessel causes the collagen within the vessel wall to be exposed to blood. Collagen is a protein found in almost every organ. Platelets in the blood adhere to collagen, and once this happens they release chemicals which make other platelets stick to them. A loose plug of platelets eventually forms in this way, and this plug may suffice to seal the vessel in small injuries. This process is aided by constriction of blood vessels.

In the next step, this loose plug anchors down a protein called fibrin. Fibrin forms from the plasma; it is the end-product of a series of reactions of the clotting factors, which are mostly plasma proteins. For example, Factor VIII is deficient in "royal hemophilia." The process is summarized in the following diagram. Fibrin is an interlacing fabric of long strands that enmeshes blood cells and more platelets within itself. It is derived from fibrinogen, which constitutes 6% of the plasma proteins. The network of fibrin strands with platelets and blood cells is called a blood clot. Note that the erythrocytes are not actively involved in clotting; they are merely trapped in the fibrin network and add important bulk to the clot. For a more detailed treatment of the blood clotting process, refer to one of the physiology texts cited in the bibliography in Lesson 1.



The formation of fibrin. The arrows marked with * mean "becomes"; the other arrows mean "activates."

The following table summarizes the known clotting factors.

Factor	Name	Chemical Nature	Function
I	fibrinogen	protein	Converted to fibrin which becomes the blood clot.
II	prothrombin	protein	Converted to thrombin which is needed for conversion of fibrinogen to fibrin.
III	platelet factor, thromboplastins	varies	These factors vary in different tissues and are needed for conversion of prothrombin to thrombin.
IV	calcium	inorganic ion	Several functions including conversion of prothrombin to thrombin.
V	labile factor	protein	Speeds conversion of prothrombin to thrombin in extrinsic system.
VI	formerly "accelerin", no name at present	?	?
VII	stable factor	protein	Speeds conversion of prothrombin to thrombin.
VIII	antihemophilic globulin	protein	Precursor of thromboplastin.
IX	Christmas factor	protein	Helps control amount of thromboplastin formed.
X	Stuart-Power factor	protein	complex
XI	plasma-thromboplastin antecedent	protein	Required for production of thromboplastin.
XII	Hageman factor	protein	Important in initiation of coagulation outside of body.
XIII	fibrin-stabilizing factor	protein	Converts soluble fibrin to insoluble fibrin by cross linking subunits.

If plasma is separated from blood cells and allowed to stand, a clot of fibrin will form; if the clot is removed from the test tube, what is left is serum. Serum is plasma minus fibrinogen and some of the clotting factors.

Vitamin K is needed for the body to make four of the clotting factors, so vitamin K deficiency can lead to poor clotting. Since these factors are made in the liver, some liver diseases (e.g., alcoholic cirrhosis) can result in poor clotting.

INFORMATION ON LABORATORY ACTIVITY 16:

TEACHING NOTES:

1. The purpose of this activity is to give the students an opportunity to determine their coagulation time and to observe blood clotting under the microscope.

2. Anticipated time: one period.

3. Since the procedure calls for two skin punctures (one in Part I and another in Part II) it may be desirable to have the students work in pairs and each make one puncture. The Materials List was made up with this option in mind.

4. The first fibrin threads may be missed by the students since they won't know exactly what to look for. It may be a good idea for the instructor or an assistant to set up a demonstration slide at the beginning of the period so that students can see these fibers in advance. (Fibrin threads may be seen even in a dried drop of blood.)

5. It might be of interest to tabulate the student results on coagulation time on the board so that the class can see the range of values. Values slightly above or below the norm should not alarm the students because there are many variables in the methodology. You might also point out that in actual clinical tests, venous blood is used rather than capillary blood for determination of coagulation time. The venous blood is believed to give more dependable results because the venous blood is less likely than capillary blood to be contaminated with tissue fluids.

MATERIALS: (for 15 set-ups)

30 sterile blood lancets

30 alcohol cotton wipe packets

15 to 30 capillary tubes

15 microscopes

15 glass-marking pencils

15 microscope slides

clock with second hand

ANTICIPATED RESULTS:

PART I: At first, minute threads of fibrin form along the edge of the drop. As time elapses, more threads appear, the threads grow thicker and longer. Eventually the threads meet, branch and combine to form a network. The threads appear as black lines amidst the red blood cells and the plasma.

PART II: Coagulation time: about two to six minutes for this procedure. In general, the time depends on the composition of the tube and the procedure used.

ANSWERS TO DISCUSSION QUESTIONS:

1. On the microscopic level, strands of fibrin form when blood clots. On the molecular level, molecules of fibrin aggregate to form the fibrin strands that are seen upon microscopic examination. (The student answers might mention fibrinogen, thrombin, etc., based on ST-16.)

2. Answers will vary.

3. The RBC's take on a shriveled-up appearance (crenation) as time passes. This is explained by the fact that the plasma surrounding the cells evaporates and becomes more concentrated in salts, etc. Then osmotic pressure causes water to be lost from the RBC's to the surrounding plasma. As the RBC's give up water to the plasma they shrivel up.

LESSON 17: BUFFERS AND pH REGULATION

RATIONALE:

One of the most important homeostatic systems in life is related to pH control. Lesson 17 considers how pH is controlled by the body. The role of buffers is emphasized. In LA-17, the students find that the pH of a bicarbonate solution changes only slightly as acid or base is added to it. The concept of a buffer is developed from this laboratory finding. In ST-17, the relation of homeostasis to pH control is considered. A self-quiz is provided for student review of pH. The rest of the lesson considers how a buffer works and discusses two other homeostatic regulators of body pH (the respiratory system and the kidneys). Lesson 18 provides further laboratory experience with pH and buffers.

OBJECTIVES:

The student will:

- determine which of several solutions is a buffer when provided with several unknown solutions, pH paper, a known acid and a known base.
- give at least two examples of blood buffers.
- state the relation between pH and acidity.
- explain how a buffer solution resists a pH decrease when acid is added and resists a pH increase when base is added.
- list at least one homeostatic device, other than buffering, used in the body for regulation of pH.

SEQUENCE: LA-17; ST-17

SUGGESTIONS:

1. This lesson provides an opportunity to review and pull together material on pH and acidity treated in several widely spread lessons. pH was defined and first treated in Unit I in Lessons 29 through 32. At that time, pH was related to acidosis, alkalosis and respiration. In Unit II, the structure and nature of organic acids was introduced (Lesson 15). The current emphasis in Lessons 17 and 18 is on pH and blood buffers. The treatment is more qualitative than quantitative.

In Biomedical Mathematics, Lessons 14 through 19 review pH in a more quantitative way. These lessons include many calculations involving pH and dissociation of acids. They lead to the application of quadratic equations to pH problems. Science Lessons X and Y are "floating" lessons intended to follow Mathematics Lessons 15 and 17, respectively. They provide laboratory applications of the concepts developed in Mathematics.

2. This lesson also provides a chance to recycle the important concept of homeostasis. Homeostasis was first considered in Unit II, Lesson 14. A few examples of homeostasis are reviewed in Section 17-1. You may wish to provide others for discussion.

3. Because your students may be at different levels in terms of retention of pH concepts, a self-test is provided to encourage review as needed. This exercise may be more effective if you allow time for the students to do the self-test and review and then give a short quiz on pH.

4. The sequencing suggested is LA-17 followed by ST-17. The purpose of this organization is to allow the students to "discover" the nature of a buffer in the laboratory before being told about buffers. However, it may be useful to review the concept of pH before the activity. You could, for example, have the students work on the self-quiz (Section 17-2) before LA-17 and then complete ST-17 after LA-17.

5. Several of the problems in the self-quiz (Problems 1, 4, 5) are amenable

to checking in the laboratory and would require little laboratory preparation. Students could check their answers to Problems 1, 4 and 5 experimentally.

6. CO_2-bicarbonate buffer was chosen to illustrate buffers because it is an important blood buffer and because we refer to it so often in this curriculum. We emphasized the equilibrium

$$H_2O + CO_2 \rightleftharpoons H^+ + HCO_3^- \qquad (1)$$

However, this buffer system is actually more complex. Bicarbonate ions can dissociate further

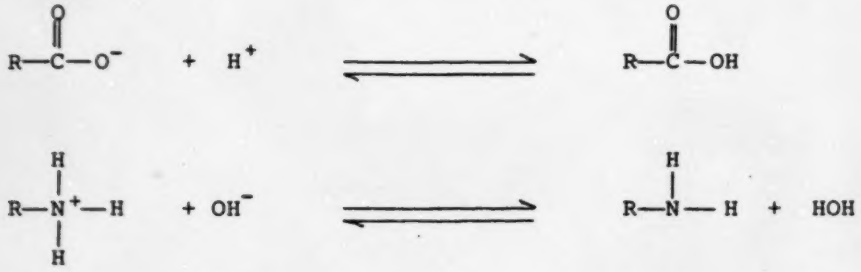
$$HCO_3^- \rightleftharpoons H^+ + CO_3^{--} \qquad (2)$$

But this reaction is of negligible importance at body pH's.

7. If you review CO_2, respiration and pH, it might be useful to have the students reread the case history on hyperventilation (Unit I, ST-29) and the subsequent discussion in ST-31.

8. In reference to Equation 1 above, we pointed out in Section 17-3 that addition of H^+ causes the formation of more $H_2O + CO_2$. This is a good example of Le Chatelier's principle, which was treated in the previous unit, Lesson 28. It would be good to remind students of the basic principle.

9. Section 17-3 mentions briefly that proteins are important blood buffers. This will be shown experimentally in LA-18. Proteins act as buffers because of carboxyl and amino side chains (See Lesson 13). These side chains buffer because of the following equilibria.



Note that the ability of proteins to act as buffers is related to their charged groups. Thus a discussion of proteins as buffers and protein migration in electrophoresis can be related.

10. Section 17-4 mentions the contribution of the kidney to pH regulation. The physiology of the kidney will be discussed in some depth in Lessons 19 to 22.

INFORMATION ON LABORATORY ACTIVITY 17:

TEACHING NOTES:

1. The purpose of this activity is to enable the students to "discover" that buffer solutions resist a change in pH.

2. Anticipated time: one period.

3. The pH of the solutions is measured with Hydrion pH paper. According to the manufacturer, this paper is accurate on buffered and slightly buffered solutions only. It reads low on non-buffered solutions. This means that the pH readings on the water and NaCl solutions will not be accurate. This discrepancy however, will not affect the overall results of the activity because it is the change in pH that is the important aspect of this activity.

4. The manufacturer suggests that the pH paper can be used either by dipping it into the solution or by placing a drop of the solution on the paper. The instructions in LA-17 suggest the latter method: to place small pieces of pH paper on paper

towels and drop the solution onto the pH paper. You could also use spot plates if they are available. In this option, the students would place the pH paper in the spots and drop solution on them.

5. The main emphasis here is not on quantitation. The activity was designed so that the students can discover how a buffer works.

MATERIALS: (for 15 set-ups)

20 g sodium hydroxide (NaOH)
86 ml HCl, concentrated
16.8 g sodium bicarbonate ($NaHCO_3$)
11.7 g sodium chloride (NaCl)
45 test tubes, 16 x 125 mm
45 pipets, 1-ml
15 pipets, 10-ml
60 beakers, 50-ml
15 beakers, 150-ml
30 medicine droppers
15 test-tube racks
15 glass-marking pencils
pH paper, Hydrion, range 1 to 11
graph paper

PREPARATION OF REAGENTS:

Sodium hydroxide solution, 1 M: 20 ± 0.1 g NaOH + sufficient water to make 500 ± 10 ml solution.

Hydrochloric acid, 1M: Add 86 ± 1 ml concentrated HCl to 800 ± 25 ml of water, then dilute to 1000 ± 10 ml. Dilute 200 ± 10 ml of this solution to 2000 ± 25 ml (0.1 M) for use in LA-18.

Sodium bicarbonate solution, 1M: 16.8 ± 0.1 g $NaHCO_3$ + sufficient water to make 200 ± 10 ml solution.

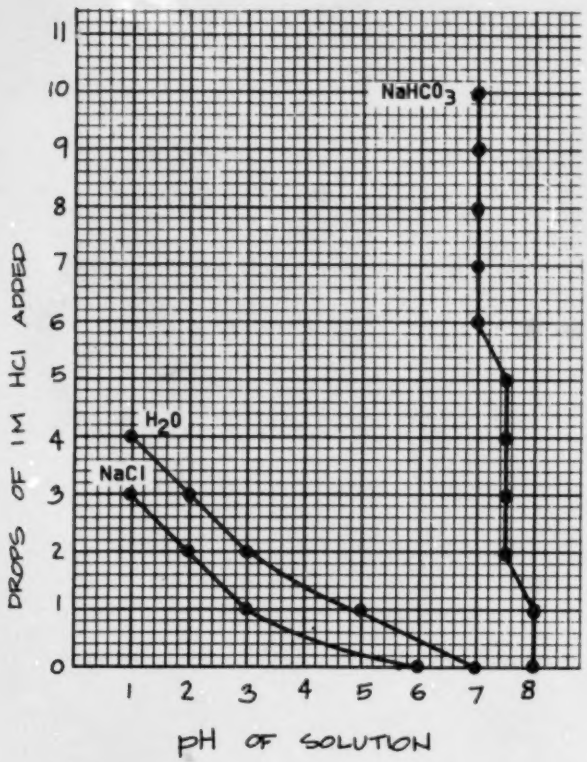
Sodium chloride, 1M: 11.7 ± 0.1 g NaCl + sufficient water to make 200 ± 10 ml solution.

ANTICIPATED RESULTS:

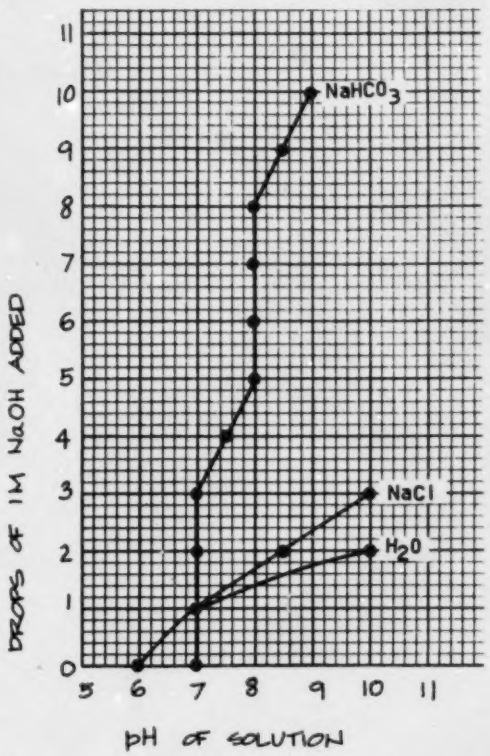
	pH of solution		
Number of drops of HCL added	H_2O	NaCl	$NaHCO_3$
0	7	6	8
1	5	3	8
2	3	2	7-8
3	2	1	7-8
4	1		7-8
5			7-8
6			7
7			7
8			7
9			7
10			7

	pH of solution		
Number of drops of NaOH added	H_2O	NaCl	$NaHCO_3$
0	6	6	7
1	7	7	7
2	10	8-9	7
3		10	7
4			7-8
5			8
6			8
7			8
8			8
9			8-9
10			9

Graph for the data cited for HCl:



Graph for the data cited for NaOH:



ANSWERS TO DISCUSSION QUESTIONS:

1. See "Anticipated Results" section. The sodium bicarbonate curve differs from the curves for water and the NaCl solution in that a much greater quantity of acid or base must be added to the sodium bicarbonate solution to cause a comparable change in pH. The sodium bicarbonate solution is, therefore, the buffer.

2. With our data, the slope for sodium bicarbonate is -10 drops per pH unit based on addition of HCl. The corresponding buffer value is -(-10) or 10 drops per pH unit. The buffer value based on NaOH addition is 5 drops per pH unit.

3. If a small amount of acid or base is ingested or produced in metabolism, the pH of the blood will not change significantly because of the presence of buffers.

LESSON 18: (A) BUFFERS IN THE BLOOD

(B) REVIEW

RATIONALE:

In LA-17 the students found that some solutions possess the capacity to resist a change in pH when acid or base is added to them. They were then told that such solutions are called buffers. Then in ST-17 the importance of buffers in maintaining physiological homeostasis was considered. LA-18 allows the students to compare the relative buffering capacities of a number of different blood components. The activity also reinforces the laboratory techniques of titration and use of the pH meter.

OBJECTIVES:

The student will:

- list at least two laboratory methods for demonstrating the presence of a buffer in a solution.
- compare and contrast these methods in regard to imprecision.

SEQUENCE: LA-18; Review

SUGGESTIONS:

1. It is desirable to get LA-18 started as early as possible to permit enough time for all the students to work with the pH meter. It would be a good idea, though, to spend a few minutes before the activity reviewing the relation between LA-17 and LA-18. You might begin by discussing the limitations of LA-17 (lack of precise measurement of volume of acid or base added, difficulty of judging pH using pH paper). Perhaps the students could suggest ways to improve the experimental design based on previous experience (use of a buret for addition of acid and base, use of a pH indicator instead of pH paper, use of a pH meter). Such ideas would provide a smooth transition from LA-17 to LA-18.

2. The technique of titrating an acid to an endpoint and the use of a pH meter have been treated in detail in Unit I, LA-29 and 30. You may wish to have the students reread the introductions to these activities before performing LA-18.

KEY--REVIEW SET 18:

1. Transport of hormones; regulation of body pH (buffering); immunological protection; control of blood-clotting; temperature control.

2. Red blood cells--transport of oxygen to cells throughout the body and transport of CO_2 from body cells to lungs. (Most of the CO_2 is transported by blood plasma.)

Neutrophils--phagocytosis.

Lymphocytes--production of antibodies.

3. Shape--doughnut-shaped or biconcave; interior--no nucleus.

4. The blood smear provides information on the concentration of each kind of blood cell in plasma. The presence of abnormal cell types may also be sought. The results are diagnostic for a number of important diseases such as leukemia, mononucleosis and anemia.

5. Cells (intracellular fluid), circulatory system (plasma), interstitial space (interstitial fluid).

6. Edema is the accumulation of excess fluid in the interstitial spaces. Edema may result from heart disease, malnutrition (kwashiorkor), or injury (localized edema).

7. The direction of migration depends on whether the net charge is positive or negative. The distance is determined by the quantity of the net charge and the mass.

8. Electrophoresis is used clinically to study plasma proteins. After electrophoresis, the proteins are stained. From the intensity of a stained protein band, the concentration of that protein (or group of proteins) is determined. Abnormal concentrations of one or more protein fractions have diagnostic implications for a variety of conditions.

9. An antigen is a substance that is recognized as "foreign" to the body. An antigen may be a protein, but it does not have to be. An antibody is a protein made by the lymphocytes in response to an antigen. Most antibodies are gamma globulins.

10. When a toxin acts as an antigen, the antibody produced is called an "antitoxin." An opsonin is an antibody that attaches to a pathogen and prepares the pathogen for phagocytosis by neutrophils and other white blood cells.

11. Both kinds of immunity employ antibodies to protect a person from a disease. Active immunity involves antibodies produced by an individual while passive immunity involves antibodies made by some other person (or organism) and injected into an individual. Passive immunity provides a short-term protection, while active immunity last longer.

12. Production of lymphocytes, transport of fats and fat-soluble vitamins from the digestive tract to body cells, maintenance of proper distribution of proteins and water in body.

13. The enzyme thrombin is made from an inactive protein (prothrombin). Thrombin helps to convert fibrinogen (a soluble plasma protein) to fibrin (an insoluble protein), which makes up the fibers of a blood clot.

14. Blood vessels constrict, platelets aggregate and stick together at the site of the opening, a blood clot forms.

15. Answer should discuss buffering, respiratory rate, urine production.

16. Proteins, bicarbonate, phosphate, citrate, lactate, etc.

INFORMATION ON LABORATORY ACTIVITY 18:

TEACHING NOTES:

1. The purpose of this activity is to investigate the buffering capacities (buffer values) of several constituents of blood.

2. Anticipated time: one to two periods.

3. The preparation requires adjusting several solutions to a pH of 7.4. You may do this with the pH meter by adding 0.1 M HCl to lower the pH or 0.1 M NaOH to raise it. It is not necessary to achieve a pH of exactly 7.4; this would take too much time. Aim at being within one drop of 0.1 M HCl or 0.1 M NaOH of the desired pH. It will take only a few drops of acid or base to adjust the pH of the water, sodium chloride and phosphate solutions; more may be necessary for the sodium bicarbonate and especially the albumin solution.

4. One point worth emphasizing is that the blood components tested in this activity are deliberately at concentrations approximating the normal range in blood plasma. However, the bicarbonate buffer in blood is really a mixture of bicarbonate and CO_2 (from respiration). For simplicity, no attempt was made to duplicate the physiological bicarbonate buffer in the activity, but it should be recognized that dissolved CO_2 is an important part of the physiological bicarbonate buffer. If students are bothered by the inaccurate comparison of bicarbonate buffers, they could perform additional titrations in which they expire air through the bicarbonate solution prior to titration. This will not provide the exact concentration of CO_2 in blood plasma, but it would indicate how added CO_2 affects a bicarbonate buffer.

5. Caution the students not to waste the albumin solution. It is fairly expensive.

6. If you have a limited number of burets, the class can be divided into teams. The Materials List has been compiled with teams of three students in mind.

7. It might be advisable to fill the burets with 0.1 M HCl prior to class.

8. To facilitate the logistics of getting all the groups to complete the pH meter activity, standardize the pH meter against a buffer before class. Since the endpoint of the titration is pH 4.0 the buffer used to standardize the meter should also be in this range. General information on standardization of the pH meter is included in the BIP Manual and in LA-30, Unit I.

9. The pH meter set-up should be ready for use at the beginning of the laboratory period, so that a group of students can start working with it immediately. The probe should be immersed in a beaker of water. Emphasize that each group should move to the pH meter immediately when they are asked to do so to avoid bottlenecks. If there are ten groups to be processed through the pH meter activity in one period, each group will have only about five minutes to work with the instrument. (Of course, you could also extend the activity to more than one period.)

10. This activity provides an opportunity to reinforce the techniques used for error analysis in conjunction with LA-20 (the ascorbic acid titration) of Unit II. If you elect to do such an analysis, you may wish to review LA-20, Unit II and Supplementary Sections Y and Z of the Mathematics Text, Unit II, or collaborate with your colleague teaching Biomedical Mathematics.

11. It is not absolutely necessary to use distilled water to rinse the pH probe in this activity. Tap water may be used for rinsing between readings. However, when storing the probe for any period longer than several days, distilled water is recommended for soaking (particularly if the tap water in your area has a high mineral content).

MATERIALS: (for 10 set-ups)

- 1.5 g sodium phosphate, dibasic (Na_2HPO_4), anhydrous
- 0.35 g potassium phosphate, monobasic (KH_2PO_4)
- 3.0 g sodium bicarbonate ($NaHCO_3$)
- 13 g egg albumin (Sigma A5253)
- 6.1 g sodium chloride (NaCl)
- 0.1 g Congo red indicator
- 2 liters HCl, 0.1 M (prepared previously, LA-17)
- 100 ml sodium hydroxide, 0.1 M (for use by instructor in adjusting pH of solutions)
- 100 ml standard buffer (pH 4.0) --for calibrating pH meter
- 10 burets, 25- or 50-ml
- 40 Erlenmeyer flasks, 125-ml
- 10 beakers, 50-ml
- 10 graduated cylinders, 10-ml
- 10 graduated cylinders, 100-ml
- 11 ring stands with clamps (one is for pH probe)
- BIP
- pH probe
- dropper bottles for indicator solution
- graph paper

PREPARATION OF REAGENTS:

Congo red indicator solution: 0.1 ± 0.01 g Congo red + sufficient water to make 100 ± 1 ml solution. (If the tap water is very acidic, you may have to adjust the pH of this solution into the 6.0 to 9.0 range so that it is red.)

Phosphate solution: 1.5 ± 0.01 g Na_2HPO_4 (anhydrous) and 0.35 ± 0.01 g KH_2PO_4 + sufficient water to make 100 ± 1 ml solution. Dilute 10 ± 0.1 ml of this solution to 1000 ± 10 ml with water. The phosphate solution may be stored for several weeks.

Sodium bicarbonate solution: 3.0 ± 0.01 g $NaHCO_3$ + sufficient water to make 1500 ± 25 ml solution.

Sodium chloride solution: 6.1 ± 0.01 g NaCl + sufficient water to make 1,000 ± 25 ml solution.

Albumin solution: 13.0 ± 0.01 g egg albumin + sufficient water to make 250 ± 10 ml solution. Store in the refrigerator. Dry albumin may be kept indefinitely. The solution of albumin may be stored for several weeks.

Special water solution: Add dilute HCl or NaOH to approximately one liter of tap water to obtain a pH of 7.4 ± .1.

ANTICIPATED RESULTS:

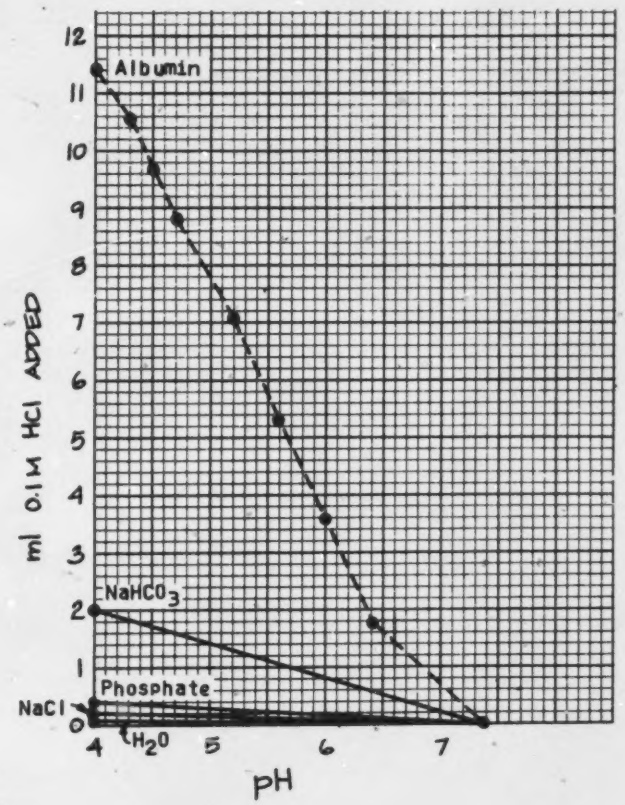
		Solution			
		NaCl	water	phosphate	$NaHCO_3$
1st Run	volume of 0.1 M HCl, ml	0.25	.03	0.40	2.00
2nd Run	volume of 0.1 M HCl, ml	0.25	.03	0.40	2.10
Average volume of HCl, ml		0.25	.03	0.40	2.05

No. drops 0.1 M HCl added	Buret readings	Volume of 0.1 M HCl added	pH of albumin solution
0	24.5	0	7.4
40	26.3	1.8	6.4
80	28.1	3.6	6.0
120	29.8	5.3	5.6
160	31.6	7.1	5.2
200	33.3	8.8	4.7
220	34.2	9.7	4.5
240	35.1	10.6	4.3
260	35.9	11.4	4.0

Volume of 0.1 M HCl required to bring pH down to 4.0 = 11.4 ml.

Buffer Values (in ml of 0.1M HCl per pH unit):

Albumin	3.4
$NaHCO_3$	0.59
Phosphate	0.12
NaCl	0.07
H_2O	0.009



ANSWERS TO DISCUSSION QUESTIONS:

1. Albumin > $NaHCO_3$ > phosphate > NaCl > H_2O. There could be much more of a substance in cells than in the blood.

2. Blood proteins other than albumin (e.g., globulins, fibrinogen), citrate, lactate, acetate, etc.

3. The red color of hemoglobin would obscure the endpoint.

4. That titration involved testing a series of dilutions of one of the solutions against a fixed concentration of the other solution. Also it involved a precipitate rather than a color change.

5. pH paper (wide range) - can be read to the nearest pH unit.

Congo red titration - reproducibility about ± 2 drops of titrant (about ± .06 ml).

pH meter can be read to the nearest .1 pH unit.

Although the comparison of the three methods is not straightforward, the method involving titration or a pH meter is corsidered to be far more precise than the one using pH paper.

6. The following factors should be considered.

a. Uncertainty associated with buret (inaccuracy of buret, scale-reading imprecision, endpoint uncertainty).

b. Uncertainty associated with graduated cylinders (scale-reading imprecision, inaccuracy of cylinder).

c. Uncertainty of pH meter (scale-reading imprecision, inaccuracy of meter).

d. Uncertainties involved in preparation of solutions.

LESSON 19: (A) THE KIDNEYS AND EXCRETION

(B) EFFECTS OF DIET ON URINE PRODUCTION

RATIONALE:

The kidneys are central to the excretory system and also play a key role in homeostasis. Malfunction of the kidneys may cause a number of diseases including hypertension. ST-19 introduces the kidneys and their functions. In LA-19, the students begin a study of the effect of diet on their own urine.

OBJECTIVES:

The student will:

- list two key functions of the kidneys and relate these functions to homeostasis.
- describe how the kidneys and liver help to remove harmful waste products from the blood.
- outline how the hypothalamus, pituitary gland and kidneys help to maintain the proper volume of body fluids.

SEQUENCE: ST-19; LA-19

SUGGESTIONS:

1. You may wish to demonstrate the location of the kidneys. They are partially protected in the back by the lowermost ribs and are separated by about 10 cm from one another. They are 8 to 10 cm long. Blows to the kidneys ("rabbit punches") are very painful and are illegal in body-contact sports such as boxing and wrestling.

2. In Figure 3, of ST-19, an unusual type of circulation is shown. You may wish to ask students in what way the circulation is unusual. (Answer: The blood goes from a vein into capillaries and then into another vein. This is an exception to the general pattern of blood flow, i.e., from artery to capillaries to vein. It occurs in two places in the body: the liver, as shown in Figure 3, and the pituitary gland. This kind of circulation in the liver is called a portal system. It insures that nutrients brought from the intestines are processed by the liver before being distributed to the body.)

3. You may wish to point out that when the liver makes ammonia into urea, several metabolic steps (each with a particular enzyme) are involved.

4. You may wish to expand on the brief treatment of antidiuretic hormone. Additional information on this hormone follows.

INFORMATION ON ANTIDIURETIC HORMONE:

1. Antidiuretic hormone is made by nerve cells in the hypothalamus of the brain. These cells have long thin branches called axons, which extend into the pituitary gland. The axons carry the antidiuretic hormone to the pituitary gland, where it is stored until released. Release is caused by nerve impulses from the hypothalamus. As stated in the Student Text, the concentration of salt in the body fluids is a major determinant of antidiuretic hormone secretion. Other factors include alcohol and an increased blood volume, which decrease its secretion, and pain, emotion, exercise and a reduced blood volume, which increase its secretion.

2. There is a disease caused by insufficient secretion of antidiuretic hormone by the pituitary gland. It is characterized by abnormally heavy urination, sometimes as much as 25 or more liters per day. This disease is known as diabetes insipidus. ("Diabetes" simply means "excessive discharge of urine.") Diabetes insipidus is treated by administering a purified extract of animal pituitary glands. Incidentally, this form of diabetes should not be confused with diabetes mellitus, which was considered in the Nutrition Unit.

INTRODUCTION ON ACTIVITY 19:

TEACHING NOTES:

1. The purpose of this three-day sequence is to enable the students to determine the effect of diet on urine composition and volume.

2. Anticipated time: one period (for Activity 19).

3. The students must decide whether to increase or decrease the amount of protein, salt (sodium) and/or calcium in their diets for a 24-hour period. It is recommended that you monitor the selection of diets to ensure that as many variations as possible are selected (increase protein, decrease protein, increase salt, etc.). It is also suggested that students on a high- or low-salt diet maintain a consistent intake of water (i.e., about as much water as they normally ingest). The results should show that an increase in salt consumption leads to a decrease in urine output. Another suggestion is to have students who go on high-protein diets keep a record of how much protein they eat. They could then relate the amount of urea produced to the amount of protein consumed.

4. USDA Home and Garden Bulletin #72 may be used to check the nutrient content of foods. Students who choose to alter salt intake should have access to Food Values of Portions Commonly Used (Bowes and Church) which gives information on sodium levels in foods. The assumption that sodium levels and salt levels are equivalent is not quite accurate, but it is a good approximation.

5. Be sure the students understand that no specimen is collected at the beginning of the procedure for collecting a timed urine sample. It may be well to go over the entire procedure in detail to ensure that it is performed properly.

6. A small pinch of sodium benzoate should be added to each of the specimen containers to retard chemical change in the specimens due to microbial growth. You may wish to explain this to the students. Urine samples should not be refrigerated since this causes precipitation of calcium salts.

7. If a weekend or holiday should intervene in the three-day sequence, make the appropriate adjustments in the schedule. For example, if Activity 19 occurs on a Friday, the students should start their special diets and urine collection on Sunday evening. If Activity 19 occurs on a Thursday, the students can collect a pre-diet urine sample on Thursday evening for analysis on Friday, but should not start the special diet until Saturday evening. The post-diet urine would then be collected on Sunday evening for analysis on Monday.

8. The reagents needed for the urine tests in LA-20 are extensive and may take some time to prepare. The reagents may be prepared considerably in advance, however, except for the urea solution for which there is a 48-hour limit.

MATERIALS: (for 30 students)

- 30 copies of USDA Home and Garden Bulletin 72
- 1 or 2 copies Bowes and Church, Food Values of Portions Commonly Used
- 30 urine specimen containers, with lids (180-ml)
- 15 g sodium benzoate (C_6H_5COONa)

LESSON 20: HOW THE KIDNEYS OPERATE

RATIONALE:

The general functions of the kidneys were introduced in Lesson 19. In this lesson, the mechanisms by which the kidneys perform these functions are considered. The important concept of active transport is introduced in relation to Na^+ transport in the kidney tubules. In addition, the role of the kidneys in homeostatic regulation is explored. LA-20 is a continuation of the study of the effect of diet on urine composition.

OBJECTIVES:

The student will:

- describe the anatomical relationship between the urinary tract and the circulatory system.
- list the components of a nephron.
- state that most of the water that diffuses from the capillaries to the kidney tubules is reabsorbed by capillaries.
- define active transport and compare this process with diffusion.
- state how active transport of Na^+ ions contributes to reabsorption of water.
- state the role of ATP in reabsorption of water.

SEQUENCE: ST-20; LA-20

SUGGESTIONS:

1. Figure 2B of ST-20 shows only one collecting tubule for one nephron. Actually the tubules of many nephrons fuse into a smaller number of collecting tubules and carry urine to a common collecting space.

2. Several points made in the student text may be extended.

a. There is one major location where virtually all of the water and dissolved substances in the blood leave the capillaries to enter the tubule. This is at the horseshoe-shaped region of the proximal tubule. Within this "horseshoe" is a coiled group of capillaries (glomerulus). Fluid in blood flows from these capillaries into the proximal tubule. In the rest of the capillaries in Figure 2B, fluid is reabsorbed from the tubule back into the blood.

b. Section 20-4 explains how the salt balance in the body is maintained. When too much salt is ingested, less salt is reabsorbed into the blood from the tubules, so that more is left to be excreted in the urine. The following questions may arise, however. If the walls of tubules are freely permeable to water, why doesn't water follow the salt out, by osmosis? And wouldn't this prevent the urine from becoming concentrated in salt? The answer is given in the Information on the Kidneys below.

3. Students might be interested in learning how the kidneys contribute to red blood cell production and to the control of blood pressure. Both of these functions are controlled by hormones made by the kidneys. Information on these hormones is provided below.

INFORMATION ON THE KIDNEYS:

When substances in blood such as Na^+ ions and water enter the nephrons, they are predominantly reabsorbed into the bloodstream rather than excreted as urine. However, other blood substances enter the nephron and are mainly excreted rather than reabsorbed. For example, some H^+ ions from acids can be eliminated, thus helping to maintain a normal blood pH. Some drugs such as penicillin are also eliminated, which tends to lower the concentration of these drugs in the blood. (This loss of drugs must be taken into account in designing drug therapy.)

The technical term for urination is "micturition." The first urge to micturate occurs when the bladder contains about 150 ml of urine. The maximum the bladder can hold is 500 ml or more.

Although the kidney's main function is to produce urine and thus help to maintain a constant chemical composition in the body, it is also the site of production of two important hormones.

1. The kidneys produce the enzyme renin. (This should not be confused with rennin, a stomach enzyme.) Renin acts to increase blood pressure. In some people, there is a reduced blood circulation to the kidneys resulting from atherosclerosis. This leads to an increase in renin production and renal hypertension. Renal hypertension accounts for about 5% of all cases of hypertension. Renin itself does not raise the blood pressure. It converts a globulin in blood plasma to a polypeptide, angiotensin II. The latter causes arterioles throughout the body to constrict, and this raises the blood pressure. Angiotensin II also causes the adrenal glands to secrete aldosterone, a hormone discussed in ST-20. This hormone helps to regulate the rate of Na^+ elimination.

2. A second hormone produced by the kidneys is erythropoietin, which regulates the rate of production of red blood cells. If too little of this hormone is produced, anemia may result. The anemia seen in kidney failure may be the result of an erythropoietin deficiency. Erythropoietin may also be responsible for the increased production of red blood cells that occurs at high altitudes.

Suggestion 3 above poses a few questions. The answers have to do with the collecting tubule which is the last portion of the tubules. (ST-20, Figure 2B) The tendency of water to follow salt out into the urine by osmosis is greatly diminished by the interstitial fluid surrounding the collecting tubule. This fluid is extremely salty, so water actually tends to leave the collecting tubule and enter the fluid around it, rather than remain in the urine. Therefore salt can be excreted in urine without excreting much water in urine. Urine is concentrated in this way.

One might wonder whether salt diffuses into the collecting tubule from the surrounding fluid--it does not. The walls of the collecting tubule are impermeable to salt. This is necessary for the above process to occur.

The fluid around the collecting tubule is made salty by active transport performed by other nearby sections of tubule. These sections remove sodium and chloride ions from the fluid within them and expel these ions into the interstitial fluid. (Water does not follow the salt out of these sections because they are impermeable to water.)

When too much water is drunk, it can be excreted due to the reduced secretion of antidiuretic hormone. This hormone makes the walls of the collecting tubule more permeable to water. Therefore when its secretion is reduced, the walls are less permeable. Less water therefore can leave by osmosis, so more remains in the urine.

INFORMATION ON LABORATORY ACTIVITY 20:

TEACHING NOTES:

1. The objective of this activity is to evaluate how changes in diet can affect the composition of urine. The students should gain familiarity with the principal components of urine, and will become acquainted with five clinical laboratory procedures.

2. Anticipated time: two periods.

3. The students should be encouraged to read the entire procedure before coming to class. All of the tests are quite simple, but the directions for performing them should be followed as precisely as possible.

4. There are various ways to structure this activity other than in the way suggested. For instance, the students can perform all of the tests while working in groups of two, although this will require extra time and equipment. It is also possible to store the urine overnight and run the tests for both normal and special diets on the same day. (Do not refrigerate the urine; the calcium salts will precipitate out.) With the preservative added, the specimens should last for 36 hours. A slight amount of decomposition of urea to ammonia may occur.

5. The quantities specified in the "Preparation of Reagents" section should be sufficient both for the two performances of LA-20 and for the artificial kidney sequence (Lessons 21-23).

6. It is advisable to divide the reagents into several small bottles so that the students will have easy access to the solutions.

7. Regarding the mouth-pipetting of reagents: although the phenol color reagent and the Sulkowitch reagent are poisonous, consumption of relatively large amounts is necessary to cause any ill effects. Consumption of small amounts of urine is also not harmful.

8. Pipets, if in short supply, may have to be re-used during a procedure. If this is necessary, the pipet should be rinsed out with water, and then rinsed again with the new solution before pipetting. Emphasis should be placed upon the importance of good pipetting technique.

9. Another technique that deserves emphasis is proper labeling of test samples. If this is not done accurately and consistently the results will be meaningless.

10. The procedure assumes that the students will be working in groups of four. Consequently, the Materials List has been prepared for a class of 32 students divided into eight groups. It is also assumed that one pH meter and two urinometers will be sufficient for eight groups if the instruments are shared. This should not pose problems, since the pH and specific gravity measurements take little time.

11. Information on programming and calibrating the BIP for pH measurements may be found in the BIP Manual. Just before class, the BIP should be standardized with a pH-7 buffer.

12. Tap water may contain trace amounts of ammonia. As a result of this contamination, a slight amount of blue color may be evident in the "blank" tube used for the urea test. Since the amount of ammonia contamination should be approximately the same for all tubes, the error introduced should be insignificant. Nevertheless, sources of ammonia such as ammonium hydroxide should not be present while preparing the reagents for the urea test, or while performing the procedure.

INFORMATION ON QUANTITATIVE ANALYSIS PROCEDURES:

1. Test for chloride concentration: The following calculations were used in selecting the $Hg(NO_3)_2$ concentration.

$$\left[\frac{1000\ \text{ml}}{\text{liter}}\right] \cdot \left[\frac{1.0\ \text{mg Cl}^-}{\text{ml Hg(NO}_3)_2\ \text{sol'n}}\right] \cdot \left[\frac{1\ \text{g Cl}^-}{1000\ \text{mg Cl}^-}\right] \cdot \left[\frac{1\ \text{mole Cl}^-}{35.5\ \text{g Cl}^-}\right] \cdot \left[\frac{1\ \text{mole Hg(NO}_3)_2}{2\ \text{moles Cl}^-}\right]$$

$$\approx 1.41 \cdot 10^{-2}\ \frac{\text{moles Hg(NO}_3)_2}{\text{liter Hg(NO}_3)_2\ \text{sol'n}}$$

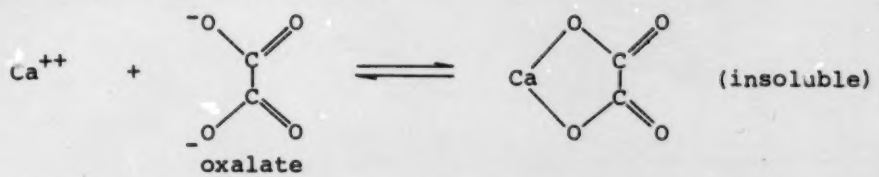
$$\approx 0.0141\ \text{M Hg(NO}_3)_2$$

The volume of $Hg(NO_3)_2$ solution that reacted with 1.0 ml of urine in our tests ranged from 0.8 to 11.7 ml.

2. Test for calcium concentration: The data we collected for the calibration line are shown in the following table.

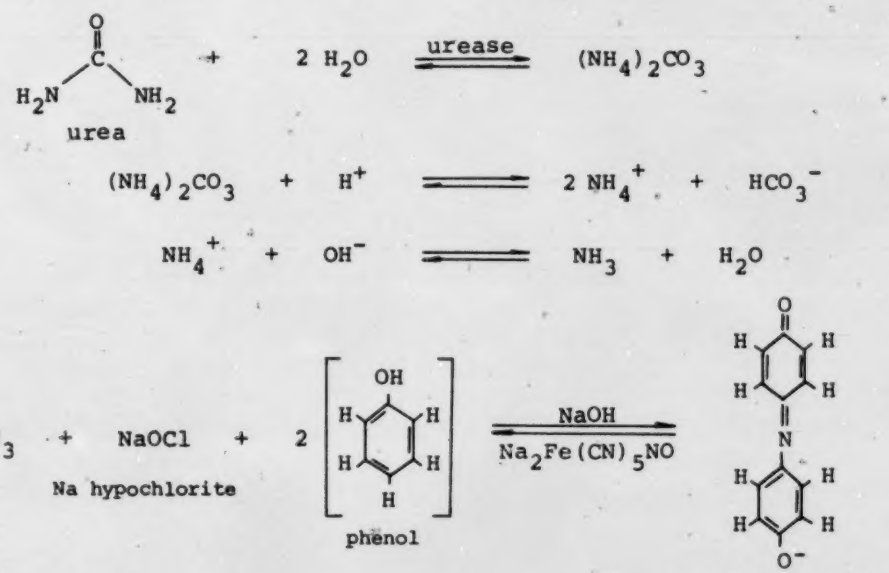
Cuvet number	Volume Ca^{++} std. (50 mg/100 ml)	Volume H_2O	%T	Absorbance	Calcium concentration
1	0.0 ml	5.0 ml	100%	0	0.0 mg/100 ml
2	1.0	4.0	90	.050	10.0
3	2.0	3.0	79	.100	20.0
4	3.0	2.0	72.5	.140	30.0

The precipitation reaction can be represented as:



3. Test for urea concentration: Of the five tests, this procedure is the most intricate, requiring the most care and precision. The students should keep in mind that two 15-minute incubation periods are necessary, and plan their time accordingly.

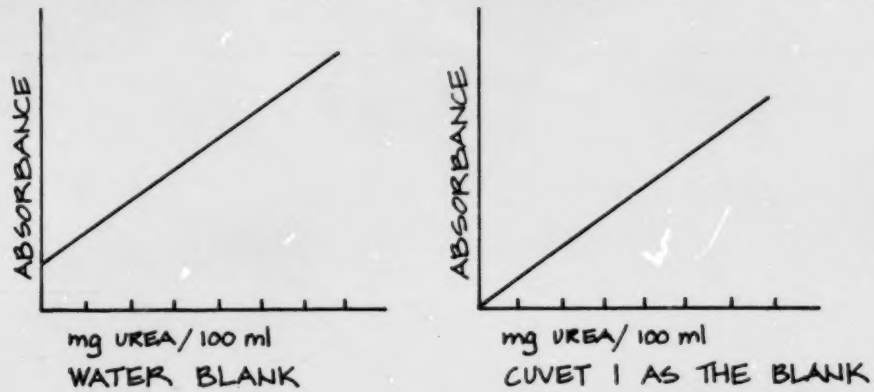
The formation of indophenol is a four-step process.



Sodium nitroferricyanide serves as a catalyst for the last step.

The incubation temperature is not critical. If an incubator or large water bath is not available, a 600-ml beaker (or other container) filled with warm water will do nicely. The temperature should be kept between 35 and 45 °C, if possible.

The use of Cuvet 1 as the blank may cause some confusion, since this tube may have some residual color arising from impurities in the urease or in the standards. If for some reason a colorimeter cannot be adjusted to null at 100%, a blank of water may be used. In this case, the graph will be moved upwards and will not pass through the origin (see illustration below). The upward deflection results because the absorbance of each sample includes the added color due to the contaminant(s).



The data collected for the calibration line should be similar to the values listed below.

Cuvet number	Volume urea Std. (10 mg/100 ml)	Volume H_2O	% T	Absorbance	Urea concentration
1	0.0 ml	1.0 ml	100%	0	0.0 $\frac{mg}{100\ ml}$
2	0.2	0.8	80	.095	2.0
3	0.4	0.6	70	.155	4.0
4	0.6	0.4	62	.210	6.0

MATERIALS: (for 8 set-ups)

- 2 urinometers
- 32 urine specimen containers, disposable, with lids (180-ml)
- 15 g sodium benzoate (C_6H_5COONa)
- 1.0 g urea [$CO(NH_2)_2$]
- 4.0 g EDTA-Na_4 (ethylenediaminetetraacetic acid, tetrasodium salt)
- 4.0 ml urease glycerol solution (60 units/ml) (Sigma 14-1)
- 10 ml colorless, liquid household bleach (e.g., Clorox)
- 5 g sodium hydroxide (NaOH)
- 5 ml phenol (C_6H_5OH), liquefied
- 0.05 g sodium nitroferricyanide [$Na_2Fe(CN)_5NO \cdot 2H_2O$]
- 6.1 g mercury II oxide (HgO)
- 62 ml nitric acid (HNO_3), concentrated
- 0.2 g diphenylcarbazone indicator
- 120 ml ethyl alcohol (C_2H_5OH), 95%
- 1.38 g calcium chloride ($CaCl_2$), anhydrous
- 16.6 g oxalic acid ($H_2C_2O_4 \cdot 2H_2O$)
- 16.6 g ammonium oxalate [$(NH_4)_2C_2O_4 \cdot H_2O$]
- 34 ml acetic acid (CH_3COOH), glacial
- 1.70 g potassium phosphate, monobasic (KH_2PO_4)--for preparation of pH-7 buffer
- 1.78 g sodium phosphate, dibasic (Na_2HPO_4) --for preparation of pH-7 buffer
- small quantity dilute hydrochloric acid (0.1 M)

160 test tubes, 16 x 125 mm, Pyrex

136 pipets, 1-ml (or fewer with rinsing)

88 pipets, 10-ml (or fewer with rinsing)

16 test-tube racks

16 graduated cylinders, 100-ml

8 burets, 50-ml (or 25-ml)

128 stoppers, cork #4

48 beakers, 50-ml

8 BIP colorimeters

BIP-pH meter

8 thermometers, 0-100 °C

9 ring stands with clamps (one for pH probe)

water bath(s)--sufficient for 64 test tubes

graph paper

PREPARATION OF REAGENTS:

Urea standard solution, 10 mg/100 ml: 1.00 ± 0.01 g urea + sufficient water to make 100 ± 1 ml solution. Dilute 2.00 ± 0.01 ml of this solution to a final volume of 200 ± 1 ml with water. Dissolve 0.2 g sodium benzoate in the resulting solution. Discard after 48 hours. (If a weekend intervenes between the pre- and post-diet tests, the solution will have to be made up twice.)

EDTA-Na_4 solution, 1%: Dissolve 4.0 ± 0.1 g EDTA-Na_4 in 350 ± 10 ml water. Adjust the pH to 6.5 with dilute (0.1 M) hydrochloric acid. Dilute to a final volume of 400 ± 5 ml with water. (If EDTA-Na_2 is used, the pH must be adjusted with dilute NaOH.) EDTA binds with divalent cations (Mg^{++}, Ca^{++}, etc.) that can interfere with urease activity.

Urease solution, 0.6 unit/ml: Dilute 4.0 ± 0.1 ml urease glycerol solution to 400 ± 5 ml with EDTA solution (prepared above). With refrigeration, this solution should keep up to 3 weeks. (A unit of urease activity is defined as that amount of enzyme that produces 1 mg of nitrogen from urea in 5 minutes at pH 7.0 at 30 °C.)

Crystalline urease may also be used (Sigma U1500--1500-3000 units/g). Dissolve 0.75 ± 0.05 g urease in 12 ± 1 ml of 5% KH_2PO_4 solution and add 13 ± 1 ml glycerol. This solution is used as the urease glycerol solution above.

Alkaline hypochlorite solution: Add 10 ± 0.1 ml household bleach (e.g., Clorox) and 5.0 ± 0.1 g NaOH to 500 ± 10 ml water. Place in an amber bottle and refrigerate. Discard after two months.

Phenol color reagent: To 5.0 ± 0.1 ml liquified phenol and 0.05 g sodium nitroferricyanide (a tiny pinch of this will do), add water to a final volume of 500 ± 10 ml. Place in a dark bottle to protect from light and label "poison." Store refrigerated and discard after 2 months.

Mercury II nitrate solution, 0.0141 M: Dissolve 6.10 g HgO in 62 ± 1.0 ml concentrated nitric acid; dilute to 2000 ml with water. Store in tightly capped dark bottle. Label "poison." Discard after 6 months.

Diphenylcarbazone indicator: Dissolve 0.2 ± .01 g diphenylcarbazone in 120 ± 1 ml 95% ethyl alcohol. Place in a dark bottle and refrigerate. The solution should have an orange-red color. If the color changes to dark red or to yellow, discard the solution. The reagent is generally stable for 2 months.

Calcium standard solution, 50 mg/100 ml: 1.38 ± 0.01 g calcium chloride + sufficient water to make 1000 ± 5 ml solution. 250 ml of this solution is sufficient for the activity. Why 1.38 g? $CaCl_2$ is 36.1% calcium by weight. Thus:

$$\frac{50 \text{ mg } Ca^{++}}{100 \text{ ml}} \cdot \frac{1000 \text{ ml}}{0.361 \text{ mg } Ca^{++}/\text{mg } CaCl_2} \approx 1385 \text{ mg } CaCl_2$$

Keep tightly capped. Discard after 6 months.

Sulkowitch reagent: Dissolve 16.6 ± 0.1 g oxalic acid and 16.6 ± 0.1 g ammonium oxalate in 800 ± 20 ml water and add 34 ± 1 ml glacial acetic acid. Gentle heating may be necessary. Then dilute to 1000 ± 10 ml with water. Label "poison." Discard after 6 months.

ANTICIPATED RESULTS:

The following table lists the results we obtained from five subjects testing five diets. Marked differences are to be expected in some cases depending upon the subject's lifestyle, diet and laboratory technique. Deviations from the values we obtained are not unusual, and should not cause any concern.

TYPE OF TEST	TYPE OF DIET									
	High Protein		High Calcium		Low Salt		Low Protein		High Salt	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
UREA mg/100 ml	*3,500	*2,900	400	500	300	500	800	800	3,000	1,800
UREA mg/day	18,550	16,124	14,800	13,400	12,685	14,400	13,440	12,480	15,990	22,680
CHLORIDE mg/100 ml	760	520	80	120	410	130	130	320	540	1,170
CHLORIDE mg/day	4,028	2,891	2,960	3,216	3,670	1,924	2,184	4,992	2,878	14,742
CALCIUM mg/100 ml	2.0	43.5	13.6	15.5	20.8	8.4	18.6	28.0	22.8	10.4
CALCIUM mg/day	10.6	242	503	415	186	124	312	437	122	131
SPECIFIC GRAVITY	1.023	1.027	1.002	1.003	1.023	1.003	1.006	1.014	1.030	1.028
pH	6.5	5.3	7.3	7.3	5.7	7.0	6.2	8.0	5.6	7.1

*Note: Ordinarily, urea levels would be expected to increase as a result of a high-protein diet. Our results indicate that this is not necessarily the case. The reasons for these results are not clear (in fact, another subject--a vegetarian--did show a marked increase in urea levels subsequent to the high-protein diet). We include these values to show that such results should not be unexpected.

Changes in diet should affect the amount of chloride, calcium and urea excreted per day, depending, of course, upon the abnormal diet selected. All values should be expressed as mass (mg) excreted per day; if the results are reported in any other fashion, they will not accurately reflect the dietary changes.

Specific gravity is principally dependent upon fluid intake. A diuretic or large quantities of water will lower urine specific gravity to near 1.00.

Dietary changes should not change the urine pH significantly except in certain special cases. Metabolism of fats produces more acid residues than the metabolism of carbohydrates, and thus results in a more acidic urine. Fasting will also lower the pH, as a consequence of the utilization of stored body fat. High-protein diets result in an increased production of phosphates and sulfates; this may lower the pH of the urine. Conversely, low-protein diets may result in urine with a higher pH.

LESSON 21: URINALYSIS, CATHETERS AND CYSTOSCOPES

RATIONALE:

The purpose of ST-21 is to explain the clinical significance of urinalysis and to consider other procedures used to study the urinary tract. The subject of urinalysis follows the series of activities in which students analyze their own urine

in relation to special diets. Lessons 22 and 23 extend the discussion of clinical problems of the urinary tract.

OBJECTIVES:

The student will:

- list at least four clinical applications of urinalysis.
- state two ways that urinalysis is used in diagnosis of diabetes.
- describe a catheter and state two medical applications of this device.
- state how the cystoscope is used clinically.

SEQUENCE: ST-21; LA-21 (read only)

SUGGESTIONS:

1. Figure 1 of ST-21 shows the molecular structures of the three ketone bodies commonly found in the urine of people with untreated diabetes. Hydroxybutyric acid is not a ketone but is classed as a ketone body because it is made from acetoacetic acid, which has a "keto" group (-C-). The conversion of acetoacetic acid to hydroxybutyric acid occurs in the liver, as does the synthesis of all ketone bodies.

2. The text states that urobilinogen is responsible for the yellow color of urine. Strictly speaking, urobilinogen is colorless. It becomes yellow when it is oxidized to the compound urobilin. Hence the darness of urine is a function of the concentration of urobilin. The dark urine seen in some cases of hepatitis is due to the presence of bilirubin, not urobilin. Bilirubin normally is converted to urobilinogen by bacteria in the intestines. However, in some kinds of liver disease bilirubin, which normally enters the intestines as part of the bile, is not secreted by the liver. Instead it backs up into the blood and enters the kidneys, darkening the urine. When bilirubin is deposited in the skin it also causes the jaundice seen in hepatitis.

3. In ancient times doctors would taste urine to aid in diagnosis. As discussed in Unit II (Lesson 32), "mellitus" means "sweet" in Latin. Since diabetics excrete glucose in their urine, the urine is sweet. This condition was distinguished from diabetes insipidus. "Insipidus" means "insipid" or "without flavor." In this condition the urine is not sweet but comparatively tasteless.

INFORMATION ON LABORATORY ACTIVITIES 21 AND 22:

TEACHING NOTES:

1. The objective of this activity is for the students to design artificial kidney models which will function, in principle, like an actual artificial kidney. During the first three periods, the students will become familiar with available materials and then design, build and test their artificial kidneys. During the fourth and fifth periods, they will be given a sample of simulated blood with a composition similar to that of blood taken from a patient with kidney failure. The students will attempt to restore the blood to its normal composition by passing it through their artificial kidneys. They will evaluate the extent of their success by conducting quantitative tests for chloride, urea and bicarbonate.

2. Anticipated time: 5 periods minimum (LA-21,22,23).

3. Since there is much to be done in this activity, we suggest that the class be divided into groups. Groups of three would be the smallest possible division (assuming a class of 30), if each group is to have access to a BIP for the tests to be run in LA-23.

4. The success of the activity relies upon a certain amount of initiative, creativity and advance preparation on the part of the student. The student should

be familiar with the overall sequence before it is started. Emphasize that time will be limited and that students will have to coordinate duties within their groups so that the time available will be used most effectively. You may wish to indicate the basis on which the models will be evaluated. (See the Evaluation of Artificial Kidney section of the Information on LA-23.)

5. The students should submit a proposal showing their planned composition of the dialysis fluid for your approval as early as possible. Check to see whether the concentrations are reasonable. You may use the following table to check their plans. If they are inside the ranges in the table, their planned concentrations should be approved. If their values lie outside the ranges shown for Cl^- and HCO_3^-, their models are likely to process the uremic blood inadequately or "overprocess" it (resulting in concentrations that are abnormal in the opposite direction).

Cl^-	100 to 300 mg/100 ml	1.6 to 5 g NaCl/liter
HCO_3^-	200 to 500 mg/100 ml	2.7 to 6.9 g $NaHCO_3$/liter
urea	0 mg/100 ml	0 g urea/liter

6. Some students may have problems figuring out how to convert desired concentrations of Cl^- and HCO_3^- to concentrations of NaCl and $NaHCO_3$, respectively. Once the desired Cl^- concentration is expressed in g/liter, the conversion factor for the chloride is approximately 1.648, as explained below.

$$Cl^-: \quad 200 \text{ mg/100 ml (desired concentration)}$$

$$= .2 \text{ g/100 ml}$$

$$= 2 \text{ g/liter}$$

$$\frac{NaCl}{Cl^-} = \frac{58.44}{38.453} \approx 1.648$$

Thus the mass of NaCl should be approximately 165% of the mass of Cl^- needed.

$$(1.65)(2 \text{ g/liter}) = 3.3 \text{ g NaCl/liter}$$

Similarly,

$$\frac{NaHCO_3}{HCO_3^-} = \frac{84.00}{61.01} \approx 1.377$$

7. The only plastic membrane that will work is cellophane film or cellophane dialysis tubing. Designs that make use of other films should not be approved. Note: dry dialysis tubing is often very difficult to open. This can be accomplished by moistening an end of the dialysis tubing with water and rubbing the material as it softens between thumb and forefinger. Eventually the end will open up.

The films may be tested in many ways. A sample of a colored solution (for instance, dilute $KMnO_4$ solution) can be placed on one side of the film, and clear water on the other (see diagram). If the water becomes colored after a reasonably short period of time, it can be assumed that the material is permeable to small molecules and electrolytes. Warn the students about the possibility of leaks.

8. The designs for the artificial kidney should also be approved as early as possible. If the proposed design has serious flaws, give the group a copy of the Troubleshooting Worksheet, and indicate that modifications must be made. If relatively small problems are noticed, it may be desirable for the students to solve these by themselves in their preliminary tests (LA-22).

MATERIALS:

HARDWARE: It will be necessary to provide all of the materials listed in the Student Procedure. The exact quantity of these items is clearly not critical, but enough should be available so that all the students will have the opportunity to test each of them. Most of the equipment should be available in the classroom. Materials that may be brought from outside of class include:

white glue (approximately 1 pint)

shellac (approximately 1 gallon)

silicone rubber cement (3 to 5 tubes, 3 oz each)

string, heavy thread (for tying off dialysis tubing)

The students will be expected to bring additional materials from home to complete their models.

PLASTICS: The following plastics, if available, should be provided when students begin LA-21. (Note: 1 mil = 0.001 inch.)

25 feet cellophane dialysis tubing, thickness approximately 1 mil, assorted sizes. Flattened widths are typically 3/8", 1", 3/4", 3"; these are available in 25', 50' and 100' rolls from Carolina Biological Supply (catalog number 68-4200).

25 feet cellophane film, width 12 to 30", thickness approximately 1 mil. This film must be "regenerated" cellulose, and not cellulose acetate. Cellophane is often treated to make it impermeable to water--uncoated cellophane is the only acceptable material. Uncoated cellophane will soften and swell considerably when it is soaked in water for a short time, but treated cellophane will not soften.

25 feet polyethylene film, width 12 to 30", thickness 1 to 3 mils. "Handi-Wrap," "Glad Wrap" or any similar polyethylene food wrap is suitable.

25 feet polyethylene "lay-flat" tubing, thickness 1 to 3 mils, flattened width 1/2 to 3".

25 feet mylar polyester film, width 12 to 30", thickness 1 to 3 mils.

Aside from the cellophane tubing and film, the types of plastics are not critical. Since polyethylene tubing and mylar film may not be readily available, other plastics may also be provided such as "coated" cellophane and saran (Saran Wrap). Of the plastics listed above, only cellophane film and dialysis tubing will work in the artificial kidney model. Other dialysis materials that can be experimented with include parchment paper and Goldbeater's skin, both available from Cenco Scientific Company.

Additional amounts of cellophane should be provided as the students learn that the other plastics are unsuitable. A 50-foot role of cellophane film should be sufficient for the entire multi-period activity.

CHEMICALS: The preparation of the dialysis fluid will require:

up to 200 g sodium chloride (NaCl)

up to 200 g sodium bicarbonate ($NaHCO_3$)

There are no absolute requirements for other chemicals during the first three days. However, a variety of reagents should be on hand so that students will be able to test their models using any practical method available. The following chemicals are recommended.

urea $(NH_2)_2CO$

potassium permanganate ($KMnO_4$)

chromium potassium sulfate [$CrK(SO_4)_2$]

calcium chloride ($CaCl_2$)

Useful solutions might include:

Ca^{++} standard solution
Sulkowitch reagent
} From LA-20

0.1 M HCl (with pH paper)

The reagents used for the analyses in LA-23 should be made available in the preceding lab period so that the students will have a chance to become familiar with the new procedures. Both the chloride and the bicarbonate tests will indicate very quickly whether or not the model is dialyzing properly.

TROUBLESHOOTING WORKSHEET

1. Is the system leakproof? Have you remembered to waterproof all materials in contact with blood or dialysis fluid? How will you prevent your connections from leaking?

2. Is there adequate surface between the two solutions? How can the surface area of the dialysis membrane be increased?

3. Have you included ways to circulate the blood and the dialysis fluid? Remember that substances will transfer across the membrane more rapidly when the fluids are in motion.

4. Have you provided for a way to introduce blood into the system? It may be desirable to have an outlet as well as an inlet connection.

5. Make sure you have a sufficient volume of dialyzing solution in your bath. For instance, if you have 400 ml of dialysis fluid, you will not be able to reduce the urea level adequately unless you provide for a way to change the dialyzing solution periodically. Inlet and outlet connections may be advisable here also.

6. It is essential to keep the volume of the blood chamber small enough. Remember that your uremic blood will have a volume of only 100 ml, and that if the volume of the blood chamber is greater than this, problems may arise.

LESSON 22: THE ARTIFICIAL KIDNEY

RATIONALE:

The purpose of ST-22 is to introduce the students to a sequence of activities in which they will design an artificial kidney model. The model will treat simulated uremic blood plasma and correct its composition to approximate that of normal blood plasma.

To this end, dialysis, as utilized in the artificial kidney, is introduced and discussed; and shock, a medical condition which often occurs following kidney failure, is discussed. In the context of kidney failure, kidney transplants are introduced.

OBJECTIVES:

The student will:

- list at least two conditions that lead to kidney failure.
- define uremia, dialyzing fluid, dialysis.
- describe the principles of an artificial kidney.
- state how tissue typing is used prior to kidney transplants.
- describe the condition known as "shock."
- list at least two conditions that lead to shock.

SEQUENCE: ST-22; LA-21; LA-22

SUGGESTIONS:

1. A field trip to a hospital to see a renal dialysis machine (an "artificial kidney") would be a good supplement to this lesson.

2. You may wish to point out that the term "dialysis" is not limited to the blood. It refers to any technique used to alter the concentration of substances by means of diffusion.

3. The discussion of tissue typing relates to lessons on immunology. You may find, in the current lesson, a chance to review the nature of antigens and antibodies. Note also the information on kidney transplants below.

4. The discussion of shock is appropriate here, but it is brief. The subject will be considered again in a later unit in the context of first aid.

5. The interpretation of the data in Table 2 of ST-22 is more complicated than presented. It is true that there is much support for the validity of the statement in the text about the decreased rejection rates for transplants between close relatives compared with transplants between non-relatives. However, the data of the table are not sufficient evidence for such a conclusion. The table alone is not very convincing because the kidneys from relatives are generally taken from live individuals while the kidneys from non-relatives are generally from cadavers. One could argue that kidneys from cadavers are not as viable as kidneys from living individuals.

INFORMATION ON KIDNEY TRANSPLANTS AND DIALYSIS MACHINES:

1. In organ transplants, not only does the host's (recipient's) immune system tend to destroy the new organ, but the new organ may reject the host as well. The lymphocytes from the donated organ are responsible for this event, called a "graft-versus-host" reaction. As a result, anemia, a reduced white blood cell and platelet count, loss of appetite and weight loss may occur. Immunosuppressive therapy is essential when a severe "graft-versus-host" reaction occurs.

2. Dialysis machines ("artificial kidneys") have a volume of about two cubic feet. A plastic tube carries the patient's blood into the machine, where the blood moves through narrow channels made of thin synthetic membranes with a total surface area of about one square meter. Blood moves through the machine and is returned to a vein. Around the channels is a special fluid called dialyzing fluid. In the widely used machine made by Travenol, Inc., there are 500 ml of blood in the machine at any one moment surrounded by 8 liters of dialyzing fluid; and blood flows at the rate of 150 to 400 ml per minute. (This information may be useful in the laboratory design for LA-22.)

The blood passes through the machine many times to complete the treatment. The synthetic membranes have pores approximately 24×10^{-10} meter in diameter. (A water molecule is about 2×10^{-10} meter in diameter, by comparison.)

Dialysis machines can also be used to remove accidentally ingested poisons from the blood or to tide the patient over during temporary kidney failure which would otherwise be fatal. In addition, dialysis machines are used to rid the blood of aspirin and barbiturates in overdose cases.

LESSON 23: A. THE URINARY TRACT

B. REVIEW

RATIONALE:

ST-23 reviews disorders of the urinary tract. In the laboratory, the students will complete the activity involving their artificial kidney model.

OBJECTIVES:

The student will:

- explain why the urethra and urinary bladder are more susceptible to infection than the kidneys.
- name two types of drugs used in treating urinary tract infection.
- state two sources of entry for bacteria into the urinary tract.
- list and describe at least two kidney disorders.
- describe and evaluate a model of an artificial kidney of his or her own design.

SEQUENCE: ST-23; LA-23.

SUGGESTIONS:

1. The point is made in Section 23-2 that infections of the urethra are especially common in females. The reason such infections are more common in females than in males is due, in part, to the proximity of the urethra to the anus and vagina. Fecal and vaginal bacteria may inadvertently be transferred to the urethra.

2. Infections of the urinary tract are treated in a later unit on reproduction and genetics, because such infections are commonly transmitted by sexual contact.

3. Homer Smith, who died in 1962, is still considered one of the outstanding authorities on the kidneys. In 1953 he wrote a short, philosophical book on the kidney and its evolution which might make excellent reading for interested students. The book was reprinted in 1961 as a paperback. The reference is: H.W. Smith; From Fish to Philosopher; Anchor Books, Doubleday, Garden City, New York, 1961.

KEY--REVIEW SET 23:

1. To remove waste products of metabolism; to control the volume of fluids in the body; to regulate the concentration of Na^+ and other substances in the body.

2. Regulation of blood pH; control of volume of body fluids; control of concentration of Na^+ and other substances in the body.

3. a. Kidney (or nephrons).

b. Wastes and other substances are removed from the blood.

4. Na^+ is pumped out of the tubule into the interstitial fluid. Water follows. This leads to reabsorption of water back into the blood. Thus, concentration of urine occurs.

5. Aldosterone; adrenal gland; makes the kidneys retain salt.
Antidiuretic hormone; pituitary gland; makes the kidneys produce less urine.

6. Blood; bacteria; protein; sugar; pH; ketone bodies.

7. Catheters--to sample and/or remove fluid from the urinary tract.
Cystoscopes--to examine the urinary tract (without surgery).

8. If it is desired to reduce the concentration of a substance in the blood, the dialyzing fluid bath is made less concentrated in that substance than the blood. The substance then diffuses from the region of high concentration (blood) to the region of low concentration (dialyzing fluid bath). If it is desired to increase the concentration of a substance in the blood, the dialyzing fluid bath is made more concentrated in that substance than the blood. The substance then diffuses from the dialyzing fluid bath into the blood.

9. A kidney from one person is immunologically different from that of another unless the two persons are monozygous (identical) twins. Therefore, a recipient is likely to build antibodies against (and "reject") a kidney from a donor. Tissue typing is an attempt to obtain a kidney which is immunologically similar to that of the potential recipient.

10. a. A condition caused by a significant reduction of blood pressure.

b. Heavy bleeding; myocardial infarction; severe emotional upheaval; excessive loss of interstitial fluid or sweat.

11. Kidneys, ureters, urinary bladder, urethra.

12. Through the urethra; from the bloodstream.

13. Pyelonephritis; glomerulonephritis; kidney stones, polycystic disease. (See Student Text for descriptions.)

14. To be certain to kill all infecting bacteria, including the few which may survive initial drug therapy.

15. Macrophages in the liver destroy bacteria and cell debris in the bloodstream.

KEY--UNIT REVIEW SET

1. Carying oxygen throughout the body; removing carbon dioxide and other waste products of cellular metabolism from cells, transport of hormones.

2. (a) Arteries, arterioles, capillaries, venules, veins. (b) Capillaries. (c) Arteries (and arterioles). (d) Veins (and venules).

3. (a) The speed decreases. (b) The <u>total</u> cross-sectional area of the vessels increases.

4. Slow flow gives more time for diffusion to occur.

5. Elasticity of the venous walls; valves; pressure exerted by the diaphragm during breathing.

6. The cell is more likely to shrink. The solution inside the cell has a higher concentration of water than the fluid surrounding the cell. Therefore, water diffuses from inside the cell to the outside.

7. (a) Diffusion. (b) The concentration of oxygen is greater in the blood than in the fluid surrounding the blood vessels (the interstitial fluid). Therefore oxygen diffuses from the blood into the fluid around the blood vessels. (c) Lungs.

8. The wall of the left ventricle contains much more muscle and is therefore much thicker than the right.

9. Venae cavae, right atrium, right ventricle, pulmonary artery, lungs, pulmonary veins, left atrium, left ventricle, aorta.

10. The heart valves.

11. 115--systolic blood pressure; 85--diastolic blood pressure.

12. The heart is damaged and so cannot pump normal quantities of blood into the aorta. Less blood in the aorta causes less blood pressure throughout the arteries.

13. Nerves leading from the brain to the heart stimulate the heart to increase its rate. The adrenal gland secretes the hormone epinephrin, which also increases the heart rate.

14. The stroke volume (volume of blood pumped per beat) increases.

15. Myocardial infarction can alter the QRS complex in several ways, depending upon which part of the heart is affected.

16. The P wave represents atrial activation. The QRS complex represents ventricular activation. The T wave represents the wave that readies the ventricles for the next activation wave.

17. (a) False. (b) During rest, individuals with atherosclerosis sometimes show normal ECG's, but the abnormalities may be detected by a stress ECG.

18. History of smoking, high blood pressure, high saturated fatty acid content in the diet, excess sucrose in the diet, stress, age, being overweight, lack of exercise, hereditary predisposition, being male.

19. Red blood cells (erythrocytes) and white blood cells (leukocytes). Red blood cells are far more numerous (more than 99 per cent of the blood cells are red blood cells) and are biconcave. Leukocytes are typically spherical in shape and generally larger than red blood cells.

20. (a) Hemoglobin. (b) Inside the red blood cells. (c) Anemia.

21. (a) A cell that performs a specific function. (b) Red blood cells carry oxygen. Neutrophils, one kind of leukocyte, phagocytize bacteria. Lymphocytes, another kind of leukocyte, make antibodies. Certain stomach cells produce acid, etc. (c) Differentiation is the process by which cells change in appearance and become more specialized.

22. (a) Circulatory system, interstitial space, cells. (b) Cells. (c) The circulatory system.

23. (a) When protein leaks out of the capillaries into the interstitial fluid, the concentration of protein in the interstitial fluid rises and the concentration of water falls. Therefore, more water diffuses from the blood into the interstitial space, increasing the volume of interstitial fluid. This results in the swelling. (b) Edema.

24. (a) The liquid part of blood, obtained by removing the formed elements (cells and platelets). (b) They represent five groups of proteins found in blood. (Proteins that compose a group move the same distance in electrophoresis.)

25. (a) Combating foreign "invaders" such as pathogens. (b) Neutrophils phagocytize foreign bodies. Lymphocytes produce antibodies.

26. (a) Proteins that react with and help to destroy microbes and harmful biochemicals. (b) A titer is a measure of the concentration of antibodies in serum.

27. (a) Passive immunity wears off after a much shorter time than active immunity does. (b) It is used when the danger of disease is so immediate that there is not enough time for the body to produce sufficient antibodies.

28. (a) The amount of time it takes for blood to clot outside the body. (b) Hemophilia.

29. (a) The lower the pH, the greater the concentration of H^+ ions and the greater the acidity. $[H^+] = 10^{-pH}$ M.

30. If acid (H^+ ions) is added, the equation is driven to the left so that more of the undissociated acetic acid forms. In this way, excess H^+ ions tend to be removed from the solution. If base is added (OH^-), it reacts with the H^+ ions represented on the right side of the equation, removing them and driving the equation to the right. In this way, new H^+ ions are added to the solution replacing those used to neutralize the OH^-.

31. Thirst stimulates people to increase their intake of water and increase their blood volume. The hormone ADH (antidiuretic hormone) acts on the kidneys to reduce the production of urine which tends to maintain the volume of body water.

32. (a) The property of resisting change in the body. (b) pH; volume of the blood; concentration of Na^+ and other ions.

33. (a) Water goes from the blood into the tubules. (b) By active transport, the cells in the tubule walls expel Na^+ ions into the interstitial fluid around the tubules. Then water diffuses out of the tubules, because the concentration of Na^+ ions around the tubules has been made greater than the concentration inside the tubules. The Na^+ ions and water then enter the blood by diffusion when their concentrations are higher in the interstitial fluid than in the blood. Some fluid remains in the tubules to become urine.

34. The high salt intake raises the salt concentration of the blood. Special detector cells in the kidneys monitor the Na^+ concentration of the blood. They regulate the rate of production of the hormone aldosterone by the adrenal glands. This hormone regulates the rate at which Na^+ is eliminated by the kidneys.

35. Blood--bleeding due to infection or diseased blood vessels in the urinary tract; white blood cells--infection; bacteria--infection; sugar--diabetes; ketones--diabetes; pH--diabetes, or any disease that alters the pH of the blood.

36. (a) Urea, K^+, Cl^-, uric acid. (b) See Key--Review Set 23, #8. (c) HCO_3^-.

INFORMATION ON LABORATORY ACTIVITY 23:

TEACHING NOTES:

1. In this activity the students process simulated uremic blood in their artificial kidney model for 30 minutes. Following the dialysis, the treated blood is tested for urea, Cl^- and HCO_3^- to determine the efficiency of the model.

2. Anticipated time: two periods.

3. Since the tests for Cl^-, urea and HCO_3^- are either slightly different from earlier tests or new, the calculations for these procedures should be reviewed for the student's benefit. In particular, it may be necessary to explain the dilution factors in the urea test and the proportions and calculations used in the chloride test.

4. It is possible that there will be insufficient time for all of the quantitative procedures, especially since the urea test will take at least 45 minutes. The test may be delayed for a day; the sodium benzoate added to the uremic blood should inhibit bacterial action.

5. The students do not necessarily have to dialyze all 100 ml of the blood. If 50 ml of blood are completely processed in 30 minutes, it can be assumed that 100 ml would require roughly one hour. The students should note the volume and the processing time on their final results sheet.

6. Although the procedure specifies a processing time of 30 minutes, the processing may be allowed to continue for longer periods if the analysis is postponed until the following day.

7. You may wish to caution students not to mouth pipet the barium hydroxide, which is a strong base.

MATERIALS:

From LA-20:
- 100 ml urease solution
- 200 ml alkaline hypochlorite solution
- 200 ml phenol color reagent
- 1000 ml mercury II nitrate solution, 0.0141 M
- 80 ml diphenylcarbazone indicator solution

1500 ml uremic blood:
- 13.68 g sodium chloride (NaCl)
- 2.34 g sodium bicarbonate ($NaHCO_3$)
- 4.00 g urea [$(NH_2)_2CO$]
- 1.0 g sodium benzoate (C_6H_5COONa)
- 80 ml polyvinylpyrrolidinone, K-60, 45% solution (PVP)

- 2 g barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$]
- 0.2 g Ponceau S stain
- water bath, 40 °C, sufficient for 80 cuvets
- 130 test tubes, 16 x 125 mm, Pyrex
- 60 pipets, 1-ml
- 90 pipets, 10-ml (or fewer with rinsing)
- 10 test-tube racks
- 10 BIP colorimeters
- 130 cork stoppers, #4
- 20 beakers, 150-ml
- 10 graduated cylinders, 100-ml
- 10 graduated cylinders, 10-ml
- up to 10 burets, 50-ml (or 25-ml)
- up to 10 ring stands and clamps
- graph paper

The equipment specified above is sufficient for 10 working groups of students. If the maximum number of cuvets or pipets is not available, they can be rinsed out between tests.

PREPARATION OF REAGENTS:

Barium hydroxide solution: Dissolve 2.0 ± 0.1 g barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$] in 200 ± 5 ml water. Heat moderately with stirring and allow to cool. Barium hydroxide forms a thin layer of barium carbonate on the surface of the solution from CO_2 in the air. Filter to remove the barium carbonate. The stock bottle should be tightly stoppered to avoid formation of more precipitate. If a small quantity of precipitate forms, use the clear solution for the procedure.

Ponceau solution, 0.01 g/ml: Dissolve 0.2 ± 0.05 g Ponceau S stain in 20 ± 1 ml water.

Uremic blood: Dissolve the following in 1000 ± 50 ml water: 13.68 ± 0.01 g sodium chloride (NaCl), 2.34 ± 0.01 g sodium bicarbonate ($NaHCO_3$), 4.00 ± 0.01 g urea [$(NH_2)_2CO$], 1.0 ± 0.1 g sodium benzoate (C_6H_5COONa), 80 ± 10 ml polyvinylpyrrolidinone, K-60, 45% solution (PVP), 4 ± 0.1 ml Ponceau solution. Dilute to a final volume of 2000 ± 10 ml with water. The PVP is used as a protein substitute because of its low cost. It is an extremely viscous material and takes at least a half-hour to dissolve completely. Gentle warming may help.

This solution has a composition as follows:

Substance	Concentration
Cl^-	415 mg/100 ml
HCO_3^-	85 mg/100 ml
urea	200 mg/100 ml

ANTICIPATED RESULTS:

If the artificial kidney has been constructed and tested properly, it is expected that the simulated blood would be restored to near normal levels if processing continued for perhaps one to two hours. Some designs may not provide complete dialysis, while others may dialyze the blood too well (raising the bicarbonate level too high and reducing the chloride level below normal concentrations). Other designs may contain more serious flaws that obscure any meaningful measurement of the processing. It should be kept in mind that the extent of the dialysis is only one aspect of the quality of the design.

EVALUATION OF THE ARTIFICIAL KIDNEYS:

The artificial kidneys should be judged primarily on the basis of how well the simulated uremic blood is processed. However, evaluation should take into consideration the following specific points.

1. Composition of dialysis fluid--some credit should be awarded for correct proportions of dialysis fluid ingredients, even if the kidney model does not function up to expectations. Check to see whether the recipe directions correspond with the specified dialysis fluid composition.

2. Quality of design--is the design well thought out? Are materials available and practical? How well does it approximate the design of a commercially available artificial kidney? Is the device simple to make or relatively complicated?

3. Care in construction--have potential trouble areas been adequately checked out? Take into consideration the general workmanship and the time spent in putting the artificial kidney together.

4. Speed of operation--a good artificial kidney should process the blood in one to two hours.

5. Composition of processed blood--have the quantitative analyses been carried out carefully? How well does the processed blood compare with normal blood? Was blood lost during the processing?

6. Analysis of data and answers to questions--naturally, a final evaluation of any model will depend upon all of the above points. Success is the primary indication of a good design, but credit should be given for mastery of concepts and for a good theoretical approach even if, in practice, the design falls short of expectation. It is hoped that this activity will be a learning experience--that the students will be encouraged to think analytically and to find out how well they can transform their ideas into practice.

THREE REPRESENTATIVE ARTIFICIAL KIDNEY MODELS:

The following is a discussion of three designs developed in the BICP laboratory. Many other satisfactory designs are conceivable. These examples are discussed in order to provide some guidance and to point out problems encountered in constructing an artificial kidney. All of these examples can be constructed from materials readily available in the classroom; they do not require special construction skills.

THE CONDENSER MODEL

The first kidney model developed is shown in Figure 1 on the following page. The membrane is a length of 3/8" dialysis tubing which is connected to glass tubing using carpet thread. The glass tubing on either end of the condenser is connected

with amber latex tubing to 500-ml flasks which serve as reservoirs for the uremic blood (Flasks 1 and 2). The dialysis fluid is contained in Flasks 3 and 4, which are connected to the condenser-tube jacket. Thus, the dialysis tubing is bathed in dialysis fluid. The solutions can be allowed to flow repeatedly from Flask 3 to 4 or from Flask 1 to 2 by simply raising or lowering the proper flask. The screw clamps can be used to regulate the flow speed to any desired rate. The pipets act as vents, allowing the fluid to flow at a constant rate.

The principal difficulty encountered in constructing this model is in connecting the dialysis tubing to the glass tubing and then installing this inside the condenser. The dialysis tubing should be about 4 cm shorter than the overall length of the condenser. The glass tubing is about 10 cm long. To prevent leaks, the connections must be tied firmly with strong thread using several wrappings. The dialysis tubing connected to the glass tubing can then be slid through the condenser jacket. The glass tubing is slipped through the holes in the rubber stoppers. A small amount of glycerine helps the glass tubing to slide fairly easily. The stoppers are placed over the ends of the condenser, and the glass tubes are carefully withdrawn until the dialysis tubing is straight and firm inside the jacket.

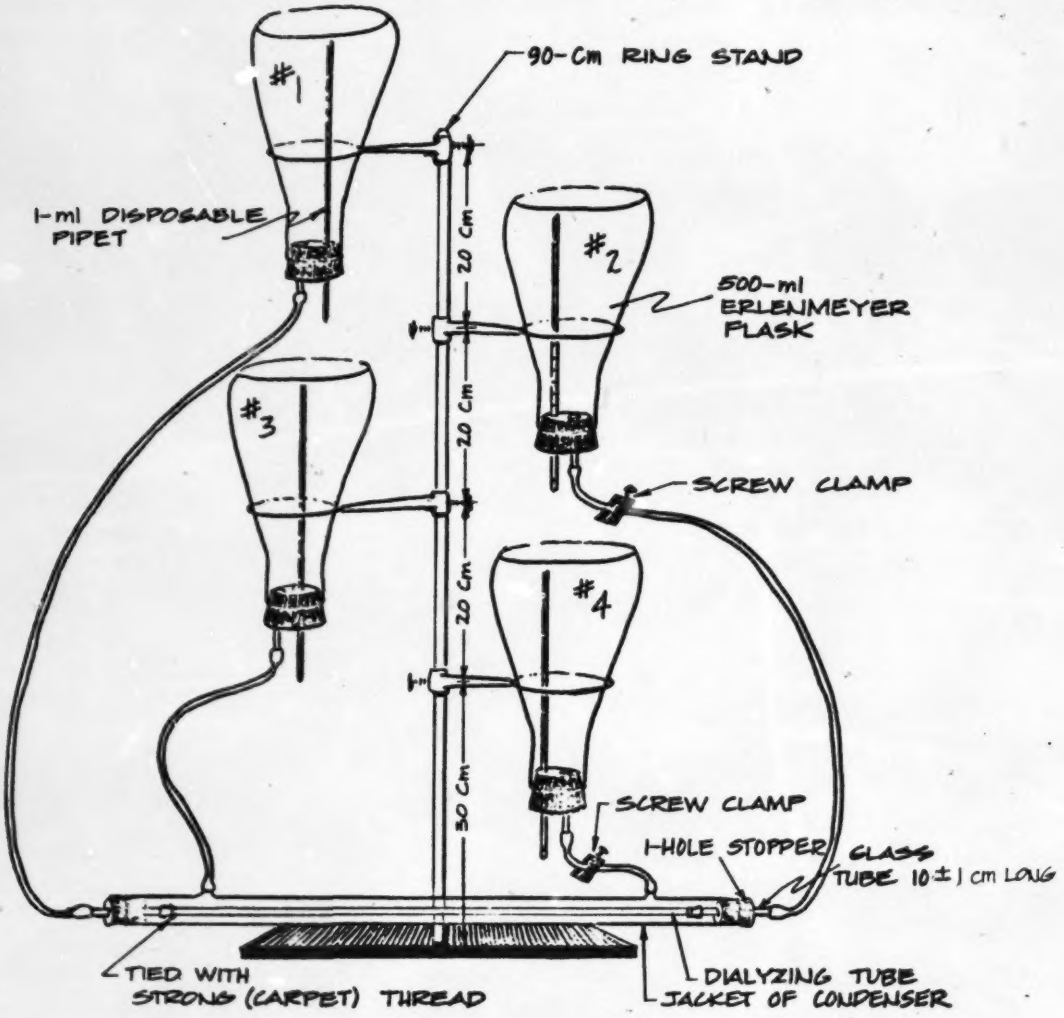


FIGURE 1

Before processing any blood with this system, it should be checked for leaks. Fill Flask 1 with a small amount (50 ml) of the liquid that will eventually be used in that flask. Gradually allow the dialysis tubing to fill with fluid. Examine the dialysis tubing at the tied joints. Any leakage at all is unacceptable.

During the processing of simulated uremic blood (UB), the flow rate of both of the fluids is adjusted to 15 ml/min. Using equal volumes of dialysis fluid and UB, the urea concentration was reduced from 200 to 100 mg/100 ml in two hours. The dialysis fluid contained no urea. Similar results were obtained for samples of chloride tested. (Note: samples of uremic blood can be taken without interrupting the main flow by merely pulling the pipet down below the fluid level of the flask that is receiving the liquid. Care must be taken to not pull out the rubber stopper or the contents of the flask will be spilled.)

This design has several minor problem areas, some of which are correctible. The blood-chamber volume can be reduced substantially using 125-ml flasks for Flasks 1 and 2. With a blood-sample volume of 100 ml and a flow rate of 3 ml/minute, the efficiency of the apparatus will be approximately doubled (with a dialysis-fluid volume of 500 to 800 ml flowing at a rate of 15 ml/minute).

The maximum efficiency of this artificial kidney is limited by the small surface area of the dialysis tubing. Also, the tubing is susceptible to leaks and breakage, especially where it is tied to the glass.

THE "SANDWICH" MODEL

This design makes use of flat plastic film (uncoated cellophane) and was found to work very well. No unusual materials or equipment were needed. Figure 2 shows an exploded view of the "kidney." It consists of the film, F, sandwiched between

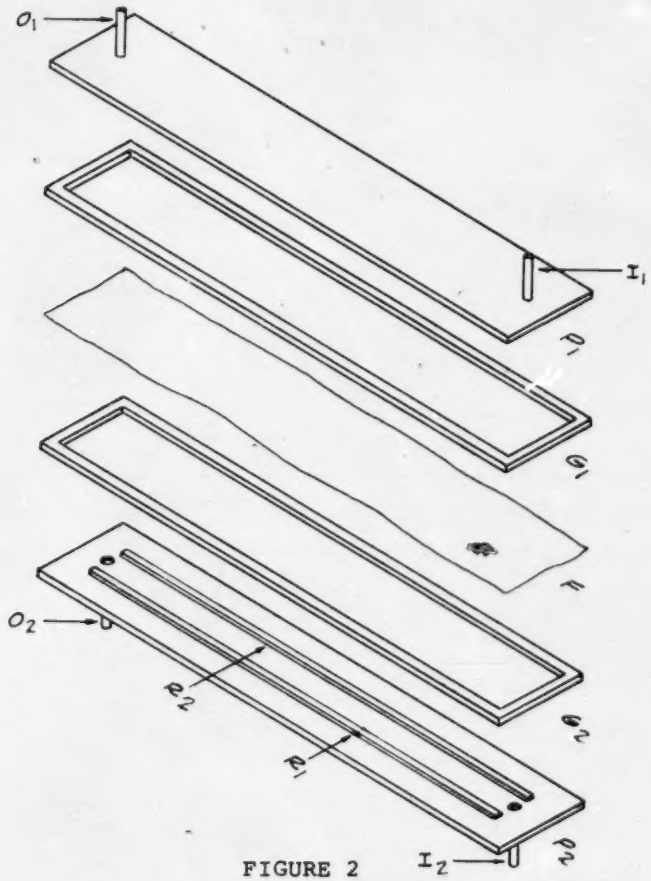


FIGURE 2

two plates, P_1 and P_2. Gaskets, G_1 and G_2, space the film slightly away from the plates so there is room for the fluid to flow. The thin ribs, R_1 and R_2, support the film and keep it from collapsing. (Their function can be best understood from the "Additional Notes" that follow.) Before the components are assembled, a thin layer of silicone rubber is used along all gasket surfaces to eliminate leaks. The sandwich is also held together by stretched tape; this is necessary because water absorbed into the cellophane may loosen the adhesion of the silicone rubber. I_1 and O_1 provide inlet and outlet connections for the dialyzing fluid, and I_2 and O_2 provide connections for the blood. Refer to Figure 3 for complete system in operation.

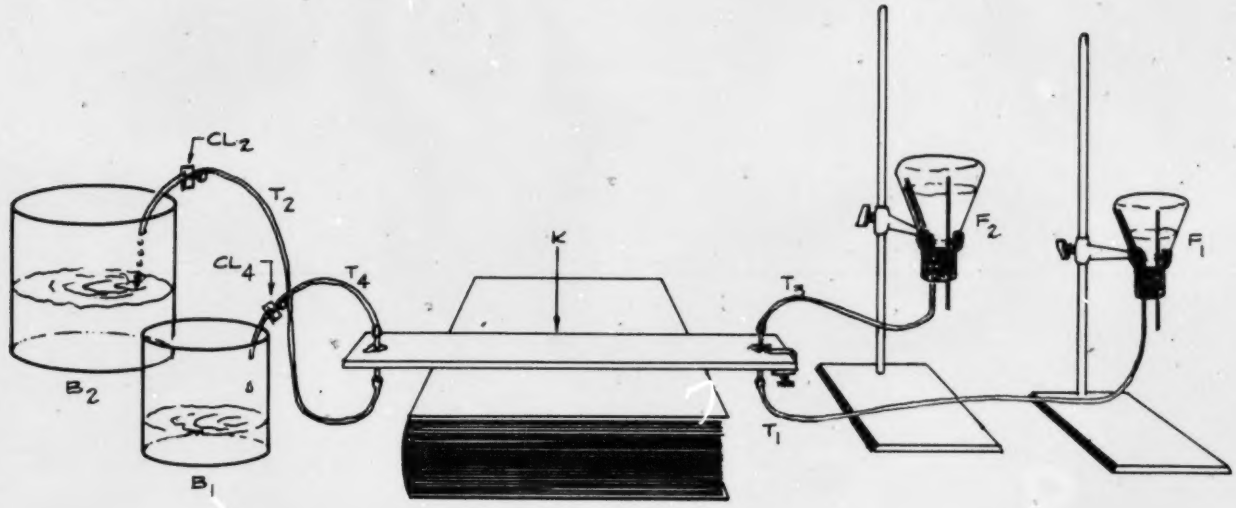


FIGURE 3

1. UB is in Flask F_1, connected to the kidney chamber containing the ribs, using tubing T_1. Screw clamp CL_2 controls the flow of UB in the outlet tubing T_2.

2. Dialyzing solution is in Flask F_2, connected to the other kidney chamber, using tubing T_3. Screw clamp CL_4 controls the flow of solution in the outlet tubing, T_4.

3. The fluid level in F_2 is kept slightly higher than the level in F_1 This insures that the membrane will be pressed against the ribs by the higher pressure in the dialyzing chamber. Fluid levels several inches higher than K will insure adequate flow rates.

4. CL_2 is adjusted so that UB flows at a somewhat slower rate than dialyzing solution. The flow rate for UB was 1 ml/min and for the dialyzing solution 10 ml/min.

5. Time permitting, UB could be recirculated several times--especially if more rapid flow rates are used. Funnels could be used instead of flasks, etc.

6. The chloride concentration of UB was reduced by 27 per cent in a single circulation (the dialyzing solution had no chloride).

Additional Notes:

•Leaks in the system necessitated recaulking the system with silicone sealant. Greater care in the original application would have prevented this problem.

•The ribs were installed in one chamber when it became apparent that it was almost impossible to maintain equal pressures in the chambers; with unequal pressures the wet membrane collapsed against the plate, and no flow would occur in that chamber.

THE COIL MODEL

This design attempts to maximize the membrane surface area available for dialysis. It uses 10 feet of 3/8" dialysis tubing wound in a coil around a plastic cylinder (Figure 4). The cylinder is prepared by cutting the top and bottom off a one-gallon polyethylene jug. The cylinder is wound with 6 coils of amber latex tubing, 1/4" outside diameter, which is secured to the polyethylene with masking tape. The dry dialysis tubing is loosely wound between the coils of latex tubing and the ends are lightly taped to the sides of the cylinder to hold them in place temporarily. Medicine droppers (without the rubber bulb) are then used to connect the dialysis tubing to the tubing leading to the flasks (Figure 5).

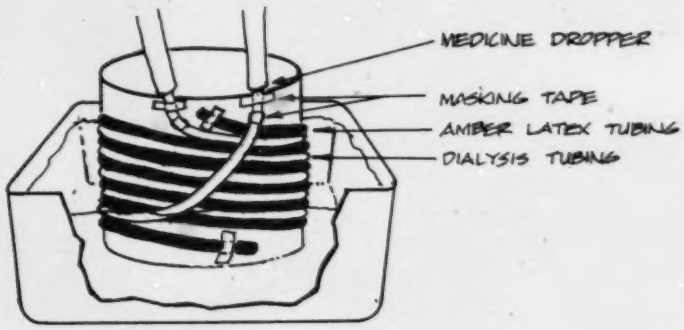


FIGURE 4

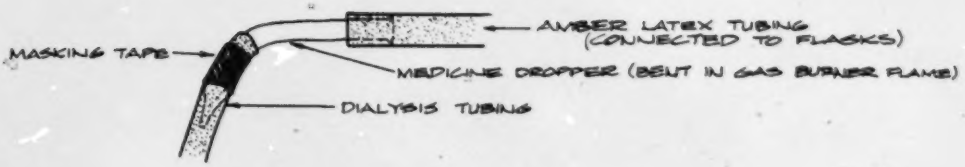


FIGURE 5

The dialysis tubing is surprisingly resilient and stands up to a certain amount of stretching. The ends must be moistened thoroughly before they are pulled over the tips of the medicine droppers.

Then 200 ml of UB are placed in one 250-ml flask, the flask is inverted, and the blood is allowed to flow slowly through the dialysis tubing. A second 250-ml flask, inverted and situated at a lower level, is used to collect the processed blood. As the tubing fills, it should be evenly adjusted around the cylinder; kinks should be eliminated as completely as possible, since they will impede the flow of fluid through the tubing. Masking tape should be used wherever necessary to keep the cellophane from twisting and kinking. (Conveniently enough, masking tape adheres very well, even when immersed in water.) As soon as the tubing is adjusted, connected to both flasks, and quickly checked for even flow, the unit may be immersed in the dialysis fluid bath (total volume of 4000 ml).

In operation, this model is capable of relatively rapid processing and displays several versatile features.

•The flow rate of the blood can be varied from 0 to 40 ml/minute, depending upon the relative heights of the flasks.

•Samples of blood may be taken during the run simply by disconnecting the tubing from the flask (when the flask has emptied at the end of a pass). These samples can be used to determine how well the dialysis is proceeding.

•Fresh dialysis fluid may be added during the run, if desired.

•Stirring of the dialysis fluid is also possible; any agitation will encourage osmosis and diffusion through the membrane. At the BICP, an inexpensive stirrer was used which consisted of a small motor connected to a paddle wheel immersed in the dialysis fluid. This contrasts with the passive circulation employed in the sandwich and condenser models.

The coil model also has some limitations. Due to the length of the dialysis tubing, the volume of blood must be at least 200 ml, which increases the processing time. The diameter of the cylinder must be greater than 6 inches (15 cm). Smaller sizes cause the dialysis tubing to kink, thereby increasing the pressure inside the membrane and restricting fluid flow.

This artificial kidney is capable of removing 90 per cent of the chloride ion from 200 ml of UB in two hours. Faster processing can be expected if the dialysis fluid is changed periodically. To process UB, the following dialysis fluid composition is recommended for the first 30 minutes of the run, using a flow rate of 20 to 30 ml/minute.

Cl^-	200 mg/100 ml
HCO_3^-	500 mg/100 ml
urea	0 mg/100 ml

After 30 minutes, the Cl^- and HCO_3^- concentrations will be near normal values, while the urea concentration will be approximately 150 mg/100 ml. To complete the run, the dialysis fluid should be replaced with a solution containing <u>normal</u> levels of Cl^- and HCO_3^- and no urea.

<u>ANSWERS TO DISCUSSION QUESTIONS</u>:

1. Answers will vary.

2. Answers will vary.

3. Answers will vary.

4. Yes. The $NaHCO_3$ in Cuvet 1 is not detected because this cuvet contains no $Ba(OH)_2$ and, therefore, the $NaHCO_3$ does not react to form a precipitate.

LESSON X: PREPARING ACID SOLUTIONS OF A GIVEN pH

RATIONALE:

Both this lesson and the next consist solely of laboratory activities. The purpose of these activities is to reinforce the material on dissociation constants and pH covered in Sections 14 through 19 of the Mathematics Text, Unit III. It is hoped that such a treatment will enhance the students' interest in the mathematics and give them an appreciation for the usefulness of such operations.

OBJECTIVES:

The student will:

- calculate the initial concentration of an acid necessary to make up a solution of a given pH.
- use the results of the calculation stated in the first objective to make up an acid solution and then test the solution to determine its pH.
- describe at least one laboratory application of the above type of calculation.

SEQUENCE: LA-X (no Student Text)

INFORMATION ON LABORATORY ACTIVITY X:

TEACHING NOTES:

1. Lessons X and Y consist solely of laboratory activities. The purpose of these activities is to reinforce the material on dissociation constants and pH covered in Sections 14 through 19 of the Mathematics Text, Unit III. The students are presented with problems which they solve on paper by performing the necessary mathematical calculations. They then proceed to the laboratory to make up test solutions and determine the pH. In this way the students can check the validity of the theoretical material they have learned. In addition, they will gain some experience with a method which may be of value to them in future laboratory work.

2. Anticipated time: 1 to 2 periods.

3. The problems that are used in LA-X and LA-Y have been taken from the problem sets in the Mathematics Text. The one used in this activity is Problem 7, of Problem Set 15. Although the answer to this problem may be found in the key to the problem set, we have included a step-by-step solution to the problem in the section on Anticipated Results, for your convenience.

4. This kind of activity can be extended by applying the same approach to other problems in Mathematics Section 15.

5. We strongly recommend that you read Sections 14 through 19 of the Mathematics Text, Unit III, and speak with the mathematics instructor, before conducting this activity and the next one. This should resolve any problems you may have with the calculations in these two activities.

6. Because the amount of acid necessary to make up the test solution in this activity is so small, it is necessary to make up a solution that is more concentrated first and then dilute it to the desired concentration. Although this is explained in the Introduction to Part II of the activity, you may wish to check on whether students understand the principle before they start Part II.

7. You may also wish to check the students' calculations (Part I) before allowing them to proceed to Part II.

8. It is very important that the benzoic acid suspension be heated (almost to the boiling point) to be sure to dissolve all the acid. If this is not done, a serious error will be introduced.

9. Clean glassware is very important in this laboratory activity. Chemicals remaining in poorly washed beakers or flasks may cause serious errors.

10. The Materials List below assumes that the students work in pairs during the preparation and testing of the solutions. You may wish to have them work individually on the calculations, however.

11. We attempted to use tap water for this activity, but were unable to obtain good results.

12. The distilled water must be adjusted to pH 7.0 before the activity is performed in order to avoid introducing error into the final pH of the test solution. (Note: Distilled water generally has a pH of approximately 5 to 6 due to dissolved CO_2 gas which forms carbonic acid.) This can be done by adding dilute NaOH and/or dilute HCl as needed. The pH meter should be used for this adjustment, standardized with a pH 7.0 buffer.

13. For student use, the pH probe should be standardized with a buffer of pH 4.0, since this is very near the desired pH of the test solutions.

MATERIALS: (for 15 set-ups)

- 0.1 M sodium hydroxide (NaOH)
- 0.1 M hydrochloric acid (HCl)
- 5.0 g benzoic acid (maximum)
- buffer solution, pH 7.0
- buffer solution, pH 4.0
- 30 Erlenmeyer flasks, 125-ml
- 15 graduated cylinders, 100-ml
- 15 pipets, 1-ml
- 15 balances
- 15 stirring rods
- 15 spatulas
- 15 gas burners
- 15 ring stands with clamps
- 15 wire gauzes
- BIP
- pH probe
- 3 to 4 liters "prepared water"

PREPARATION OF REAGENTS:

"Prepared" water: Adjust the pH of distilled water to a pH of 7.0 ± 0.1 on the pH meter. 0.1 M NaOH and/or 0.1 M HCl may be used for adjusting the pH.

Buffer solution, pH 4.0: Available from Mallinckrodt. Also, instructions for the preparation of buffers of various pH's can be found in the Handbook of Chemistry and Physics.

ANTICIPATED RESULTS:

Part I: Calculations

1. A pH of 4.2 corresponds to a hydrogen ion concentration of $(10^{-5})(10^{-.8})$ or 6.3×10^{-5} M (see conversion table in LA-X). (Note: 6.3×10^{-5} is identical to K_{eq}. This is a coincidence and could confuse some students.)

2.

		$[H^+]$	$[C_6H_5COO^-]$	$[C_6H_5COOH]$
Concentrations [M]	Initial	0	0	$z + (6.3 \times 10^{-5})$
	Equilibrium	6.3×10^{-5}	6.3×10^{-5}	z

At equilibrium, the $[C_6H_5COO^-]$ equals $[H^+] = 6.3 \times 10^{-5}$ M.

Let $z = [C_6H_5COOH]$ (undissociated benzoic acid) at equilibrium.

The initial $[H^+]$ and $[C_6H_5COO^-]$ is approximately 0.

The problem is to solve for the initial concentration of benzoic acid, $[C_6H_5COOH]$. This value must equal the sum of the dissociated + undissociated acid at equilibrium or $z + 6.3 \times 10^{-5}$.

3. $$K_{eq} = \frac{[H^+][C_6H_5COO^-]}{[C_6H_5COOH]}$$

$$z = \frac{(6.3 \times 10^{-5}\ M)(6.3 \times 10^{-5}\ M)}{6.3 \times 10^{-5}\ M} = 6.3 \times 10^{-5}\ M$$

4. $$[C_6H_5COOH]_{initial} = z + (6.3 \times 10^{-5}\ M)$$
$$= 6.3 \times 10^{-5}\ M + 6.3 \times 10^{-5}\ M$$
$$= 12.6 \times 10^{-5}\ M \text{ (or } 1.26 \times 10^{-4}\ M)$$

5. g of benzoic acid corresponding to a concentration of

$$12.6 \times 10^{-5}\ M = (12.6 \times 10^{-5}\ \tfrac{moles}{liter})(122\ \tfrac{g}{mole}) = 1537.2 \times 10^{-5} \approx 1.54 \times 10^{-2}\ \tfrac{g}{liter}$$

Part II: Testing of Solution

Our solution tested out at a pH of 4.0.

ANSWERS TO DISCUSSION QUESTIONS:

1. Answers will vary. However, if the solutions were made up correctly the pH's can be expected to be within the range of 3.7 to 4.3.

2. Deviations could be due to any of the following factors:

a. incorrect weighing of the benzoic acid,

b. incomplete dissolving of benzoic acid in preparing the initial solution,

c. careless technique in dilution procedures, such as inaccurate use of the pipet in transferring 1 ml of the initial solution to a second flask for dilution,

d. dirty glassware,

e. use of water that was not adjusted to a pH of 7.0 or was incorrectly adjusted,

f. faulty standardization of the BIP-pH meter,

g. incorrect calculations in Part I.

3. One could simply make up a solution of benzoic acid, say 1 g/liter, test the pH and then dilute the solution or make it more concentrated, as necessary, until the desired pH is reached.

LESSON Y: APPLICATION OF QUADRATIC EQUATIONS TO pH PROBLEMS

RATIONALE:

The rationale for this lesson is the same as that described in the Lesson Plan for LA-X. However, in this case the students are given the initial concentration of an acid solution and asked to determine what the pH of the solution will be at equilibrium. They are then asked to test their results in the laboratory.

OBJECTIVES:

The student will:

- calculate the pH of an acid solution when given the initial concentration of the acid.
- test the results of the calculation stated in the first objective by making up a test solution in the laboratory and determining its pH.

SEQUENCE: LA-Y (no Student Text)

INFORMATION ON LABORATORY ACTIVITY Y:

TEACHING NOTES:

1. The purpose of this activity, like that of LA-X, is to reinforce the material on dissociation constants and pH covered in Sections 14 through 19 of the Mathematics Text, Unit III. In LA-X the students were given the pH of a benzoic acid solution and requested to determine the amount of benzoic acid necessary to make up the solution. In this activity the reverse is done, i.e., they are given the initial concentration of an acid solution (ascorbic acid) and are requested to determine what the pH of the solution will be. Their results are then tested in the laboratory, using the pH meter.

2. Anticipated time: 1 to 2 periods.

3. As in LA-X, the problem used in this activity was taken from the Mathematics Text. In this case it is a combination of Problems #4 in Problem Sets 16 and 17.

4. Teaching Notes #3, 4, 5, 6, 7, 9, 10, 11, 12 and 13 from "Information on Laboratory Activity X" all apply to this activity. You may wish to refer to them before proceeding.

5. Ascorbic acid is much more soluble in water than benzoic acid. Therefore, no heating is necessary in this activity.

MATERIALS: (for 15 set-ups)

5.0 g ascorbic acid ($C_6H_8O_6$)	15 balances
0.1 M sodium hydroxide (NaOH)	15 stirring rods
0.1 M hydrochloric acid (HCl)	15 spatulas
30 Erlenmeyer flasks, 125-ml	BIP
15 graduated cylinders, 100-ml	pH probe
15 pipets, 1-ml	3 to 4 liters of "prepared water"

PREPARATION OF REAGENTS:

(See Teaching Notes, LA-X.)

ANTICIPATED RESULTS:

Part I: Calculations*

1.

		$[H^+]$	$[HAsc^-]$	$[H_2Asc]$
Concentrations [M]	Initial	0	0	1×10^{-4}
	Equilibrium	z	z	$(1 \times 10^{-4}) - z$

*Note: The numbers correspond to the steps under "Calculations" in Part I of LA-Y.

2. $8 \times 10^{-5} = \dfrac{(z)(z)}{(1 \times 10^{-4}) - z}$

3. $(8 \times 10^{-5})(10^{-4} - z) = z^2$

$(8 \times 10^{-9}) - (8 \times 10^{-5})z = z^2$

$-z^2 + (8 \times 10^{-9}) - (8 \times 10^{-5})z = 0$

$z^2 + (8 \times 10^{-5})z - (8 \times 10^{-9}) = 0$

$a = 1,\ b = 8 \times 10^{-5},\ c = -8 \times 10^{-9}$

4. $z = \dfrac{(-8 \times 10^{-5}) \pm \sqrt{(8 \times 10^{-5})^2 - 4(-8 \times 10^{-9})}}{2}$

$= \dfrac{(-8 \times 10^{-5}) \pm \sqrt{(64 \times 10^{-10}) + (32 \times 10^{-9})}}{2}$

$= \dfrac{(-8 \times 10^{-5}) \pm \sqrt{(64 + 320)(10^{-10})}}{2}$

$= \dfrac{(-8 \times 10^{-5}) \pm \sqrt{(384)(10^{-10})}}{2}$

$\approx \dfrac{(-8 \times 10^{-5}) \pm (19.60 \times 10^{-5})}{2}$

Taking only the positive solution, we have:

$$z \approx \frac{11.6 \times 10^{-5}}{2}$$

$$\approx 5.8 \times 10^{-5}$$

Thus $[H^+]$ at equilibrium is 5.8×10^{-5} M.

5. Converting 5.8×10^{-5} into a power of 10, we get $(10^{.8})(10^{-5})$ or $10^{-4.2}$. Therefore, the pH of the solution would be 4.2.

6. To obtain a solution with a concentration of ascorbic acid of 10^{-4} M, we would need

$$(10^{-4} \frac{\text{moles}}{\text{liter}})(176 \frac{g}{\text{mole}}) = 1.76 \times 10^{-2} \frac{g}{\text{liter}}$$ of ascorbic acid.

ANTICIPATED RESULTS:

Our solution tested out at a pH of 4.2.

ANSWERS TO DISCUSSION QUESTIONS:

1. Answers will vary. However, if the calculations were done correctly and the solutions prepared properly, the pH's should fall within the range of 3.7 to 4.3.

2. The sources of error in this activity are the same as those in LA-X. (See answer to Discussion Question #2 for LA-X.)